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THE PHENOMENA OF DISSOCIATION INTO S AND R FORMS OBSERVED AMONG THE BACTERIA DO ALSO OCCUR IN YEAST CULTURES

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In Collaboration with his pupil

JONAS DE SÀ VIEGAS

Introduction

IN the course of our studies on yeasts, we have often observed that, after some time has elapsed, on a certain point of a culture perfectly smooth and creamy, suddenly, a white chalky growth appears, hard and rough in appearance which further covers progressively the whole surface of the same cultures.

Such a growth is generally noticed either on the edge or in the middle of the culture and occurs specially in solid natural media, such as potato and carrot. This fact, noticed by me since 1920, when studying the yeasts fermenting the juice of *Coccos nucifera*, has eventually been stated in 1935 in the following words: "en étudiant les levures formées du jus de Cocotier, nous avons vu des cultures, crémeuses au début, devenir plus tard sèches, mates, parfois poudreuses (on glucosed agar)".

Having this year had the opportunity to study some yeasts isolated from the intestine of domestic animals, the same fact has been more frequently noticed and I have at first believed that we were dealing with a normal evolutive polymorphism, without reversible character. Soon we realised that we were perhaps in the presence of a phenomenon of dissociation and one of my pupils (Mr. A. B. Cardoso) was specially in charge to conduct some preliminary experiments with the strain E 3, isolated from the intestine of a hen, developed in potato and belonging to genus *Candida* Berkhout, as, in this strain, such a chalky growth was at the time more conspicuous than in other cultures. The subculture in potato, made from the chalky parts of the same strain gave: (1) the chalky type grew under the same chalky appearance; (2) the creamy type gave either permanent creamy cultures or creamy becoming, later on, chalky or even chalky *d'emblée* (as the method of single cell culture was not followed, it is quite possible that both types of elements were contained in the second group subcultures).

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The same fact having occurred with a striking evidence in a creamy 30 days old culture of the strain N 3 , also a *Candida* from the intestine of a white mouse—I decided to examine the mycological aspect of this growth and was so surprised to see it so different from the general aspect of a *Candida* that I thought our culture was perhaps contaminated by another yeast.

This was the starting point of the present study which, of course, required first of all, the consultation of the mycological literature on the subject.

Yeasts Mutations Observed by Early Mycologists—

Mutations of this kind have been since long ago observed by mycologists; only the interpretation of this phenomenon took a new turn in modern times. For instance. Hansen (1895) states in *Sacch. carlsbergensis* macro and microscopic modifications at low temperatures. Beyerinck (1897) obtains three types of colonies from a single strain of *Schizosacch. octosporus* and Saito the same from *Zygosacch mandschuricus*. Lepesckin (1903) keeps in pure culture a filamentous variety of *Schizosacch. mellacei* and *Schizosacch. pombe* which reproduce the same character in subsequent passages.

Definite Interpretation of these Mutations as Dissociation Phenomena—

The hypothesis about the interpretation of these mutations as dissociation phases of the yeast has been firstly drawn up by Draper (1925) who studying two yeasts, one isolated from the trush, the other from the stools of a diarrheic patient, definitively states the resemblance of these phenomena with those observed by Arkwright in bacterial dissociation.

In 1932 Seppilli and Guiso and Seppilli and Denes, working with *Sacch. cervisiæ* find two kind of colonies: R and S, the first one mycelial, the second blastoporated. The R form could be easily made reversible to the S type, when cultured on appropriate media.

Puncari and Henrici (1933) describe creamy cultures and rough cultures in *Torula pulcherrima*, identifying them as S and R forms of the same germ.

In 1934 Fabian and McCullough submitting the culture of *Sacch. cervisiæ, Sacch. ellipsoideus* and *Zygosacch. mandschuricus* to the action of various chemical substances, unsuitable temperatures and senility, obtain R and G cultures (so-called *gonidial*), easily reversible to the S type by a mere subculture in maltosed agar and T form (transitional) which could not be cultivated.

In 1934–35 appear the interesting studies of Negroni: from a strain of *Mycotorula albicans* the author obtains a R type differing from S colonies by their agglutination in a solution of Tripaflavine at 2 per 1000 and by the persistence of the capsule (?) similarly to that of the R type of capsulated

bacteriaceæ. The reversion experiments gave to the author incomplete results as there were many transitional forms—the RS type—whose colonies showed the borders not perfectly smooth and the mycological appearance of a perfect *Candida*.

In 1936, Verona describes R and S forms in *Mycotorula pinoyi* (Cast) and Kurotchkin in many strains of *Monilia* and *Candida*. Mackinnon in the same year names M the hard, membraniform, rough and crumpled cultures of *Mycotorula albicans*; they keep the new acquired characters, in subsequent passages, and are considered by the author as allied to R. forms.

Pinkerton in 1937 studying 50 strains of *Monilia* finds S, R and r forms, the last ones showing intermediate characters.

Besta (1938) found in *Mycotorula albicans* R colonies which kept their characters unchanged till the eleventh passge, returning after, suddenly, to the type S. The author emphasizes that R type is constituted mycologically by many mycelial threads with rare blastospores, while in the S type the blastospores are extremely abundant and the mycelial threads rare.

In 1939 Cavallero gives an excellent monograph with the results of his researches on various strains of *Mycotorula albicans*, whose resumé is: (*a*) the yeast, under the action of some substances unfavourable for its development, shows dissociation phenomena, giving rise to two fundamental types S and R with some transitional forms possessing intermediate characters; (*b*) the type R differs from the type S by macro and microscopical characters: in the former the blastosporic element almost disappears and is substituted by mycelial threads and some atypic formations such as chlamydospores, not seen in normal S cultures; (*c*) the biochemic activity, the pathogenicity and the non-specific agglutination in front of Tripaflavin solution at 2/1000, ClNa at 9/1000, basic fuchsin at 1/8000 and copper sulphate at O, 5/100 are also different in R and S types; (*d*) these variations are either permanent or temporary and their instability allows often the regression of the mutation variety to the primitive form.

The Dissociation Phenomena are not confined only to the Group of the Yeasts.—Extreme variations of the cultural characters have often been observed in the group of *Actinomyces*. Waksmann (1919) states them in saprophytic species of this genus. Lieske (1921) shows that the new acquired characters are permanent. Nepomniaski (1920) has grown up from a strain of *Actinomyces* three types of colonies: S with short and slender gram negative elements; O, with diphteroid Gram positive forms; and R with mycelial filaments. The same observations have been made by Triuss and Politowa (1937) and by Wright (1937).

It seems to me also that the pleiomorphic transformations of Dermato-phytes in glucose and maltose agar, with white duvet of non-reversible character, which, described by Sabouraud, have since been met with by every mycologist and hitherto considered as a degeneration, should be classi-fied among the dissociation phenomena.

Our Actual Studies with the Strain N 3—

The strain belongs to the genus *Candida* Berkhout and has been isolated from the intestine of a white mouse. A culture on Sabouraud's glucosed agar showed after 20 days a white, chalky growth of the R type, while the remaining portion of the culture was keeping the primitive characters of S type. Subcultures were made from these portions and gave the following results:

*Zone R of N 3.—*48 hours. Rough, cerebriform development, chalky; irregular borders. In the portion of the tube where the sowing has been
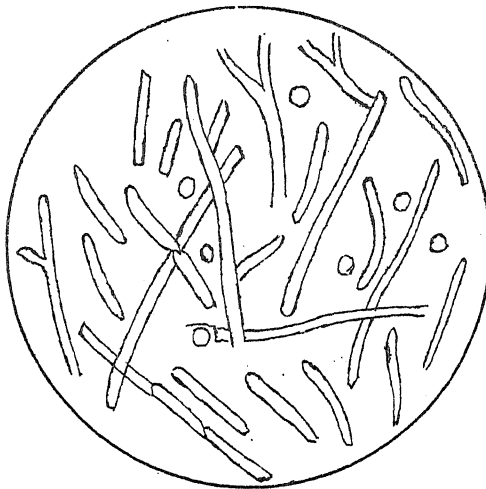
R 48 hours

slender, white chalky punctiform colonies whose coalescence gives the cere-bri-form appearance reported above.

The consistence of the growth, firstly membraniform, (48 h.) becomes more and more hard and since the beginning it is difficult to obtain an uniform emulsion of the colonies which produce clots resembling a suspen-sion of caseine.

After 8 days the growth becomes frankly verrucose and very hard to be taken off through platine wire. Under the pressure of a cover slide a segment of the culture flattens as a hard membrane.

Mycological Aspect.—Extremely filamentous with some blastospores scattered here and there, but without forming the conidial clusters so characteristic of the genus *Candida* (48 h.). Almost totally filamentous with very rare blastospores (8 days). The blastospores have entirely disappeared,
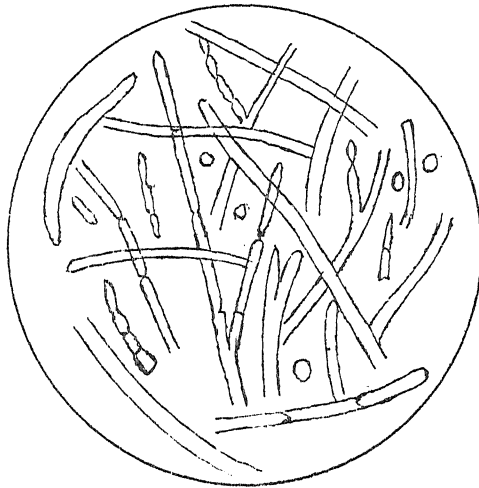
R 8 days

the mycelial threads are segmented giving rise to small quadrangular elements; many chlamydospores (30 days).
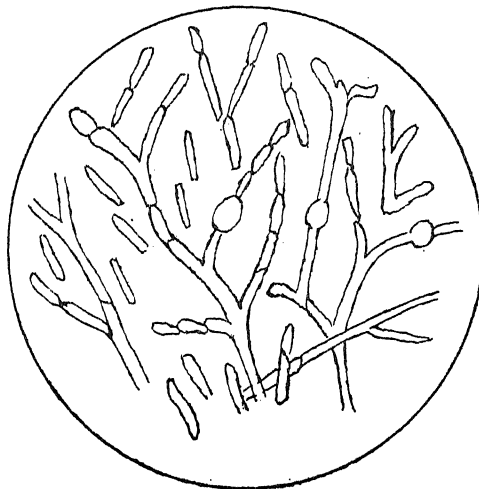
R 30 days

Zone S of N 3.—We have obtained two types of cultures:
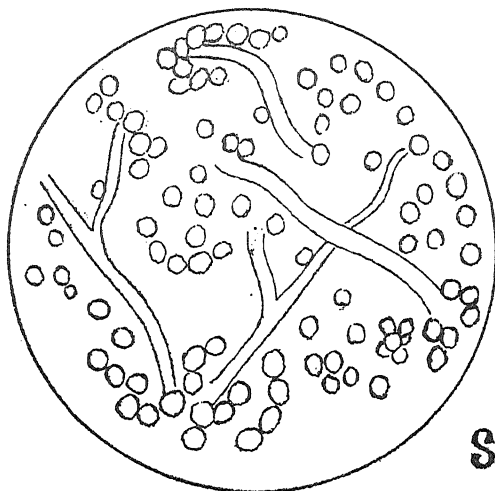
(*a*) *Type I.*—Frankly smooth, creamy, emulsifying easily.

Mycological Aspect.—Rare mycelial filaments bearing numerous clusters of conidia and independent yeast cells spread all over the preparation which is literally filled up with them.

(*b*) *Type II.*—Creamy smooth culture with some white chalky puncti-form colonies scattered here and there.

Mycological Aspect.—Nearly the same as in Type I.

The evolution of these subcultures has been followed:

(1) In type II the white chalky punctiform colonies enlarge and at the end of 8 days give rise to R growths of varying size, scattered here and there on the surface of the culture. They tend to cover the whole growth, only the borders keeping the smooth creamy consistence of the primitive culture.

(2) The cultures of type I give rise between 25th to 30th day to white chalky colonies which follow the further evolution of the R type.

(3) In some media however the S cultures of type I continued smooth during the period of observation (nearly 4 months).

The mycological aspect of the cultures is figured in the schemes illustrating this paper.

Conclusions

From the study of our yeasts, followed during some months, we are able to draw the following conclusions:

1. Cultures of yeasts show dissociation phenomena, giving rise to the two fundamental types S and R, which continue in subcultures the newly acquired characters.

2. The general mycological aspect of the R type is characterised by the predominance of the filamental mycelium over the blastospore, predominance which is often so pronounced that gives rise to the suspicion that we are dealing with a different species or genus contaminating the primitive culture.

3. The dissociation into S, R and the intermediate forms observed in yeasts and yeastlike germs seems to cover a wider field, extending to other genera of fungi.

4. The knowledge that the mycological aspect of the S and R forms in yeasts is so different one from another is particularly interesting for medical mycology, as many of the modern classifications of these fungi are mostly founded on micromycological characters. On this ground a revision of the modern taxonomy is badly required.

P.S.—From the excellent revue given by Langeron and Guerra " Valeur et Nature des Variations et dissociations de colonies chez les champignons levuriformes," *An de. Parasit. Hum. et Comp.*, 1939–40, T. XVII, No. 5 we see that Teissier has described in detail this phenomenon in 1897, " Contribution à l'étude du champignon du muguet," *Arch Med. Exp. et anat path.*, 1897, No. 3), and that before him Plaut in 1885, " *Beitrag zur systematischen Stellung des Soorpilzes in der Botanik.*," Leipzig Voigt, 1885, has pointed out the mycelial type and the yeast type of thrush fungus whose filamentation and the conditions giving rise to it are fully studied by Roux and Linossier in 1890, " Recherches morphologiques sur le champignon du muguet," *Arch. de Med. exp.*, 1890.

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NEMATODES PARASITIC IN INDIAN-COCKROACHES

By M. A. Basir

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So far very little work has been done on the nematode parasites of Invertebrates. In India this subject has remained nearly unexplored. Only a few researchers have recorded some species of Mermithidæ from Diptera and in one case an Oxyurid worm has been recorded from Scolopendra. At the suggestion of Dr. M. B. Mirza, the writer has undertaken this work and this paper is the first of a series of papers to be published on the nematode parasites of Invertebrates. The present paper deals only with the nematodes of Indian-Cockroaches. Nematodes found parasitic in Gryllidæ and certain Coleoptera are in the course of study.

A very large number of Cockroaches belonging to different species were dissected. Some of them were found to be generally infected with one or more than one species of nematodes while others did not reveal any infection at all.

(1) *Periplaneta americana* is extensively used for laboratory work in India. More than hundred specimens of this species were dissected and nearly all of them, with the rare exception of one or two, were found to be infected. On identification it was revealed that there were only three different species of nematodes parasitic in these Cockroaches. In one case a single Cockroach harboured all the three different species of nematodes. All these belong to the sub-family *Thelastomatidæ*. On further examination it was revealed that *Hammerschmidtiella diesingi* Chitwood, 1932, so common in *Periplaneta americana* in Europe and America was present in this country also. This nematode was first described by Hammerschmidt in the year 1838 as *Oxyuris diesingi*, redescribed by him as *Oxyuris blattæ orientalis* in 1847, and again described by several authors under different names all of which fall synonym to the new name given to it by Chitwood (1932). Of the other two species, one represents the Genus *Thelastoma* Leidy, 1849,

and has been named *Thelastoma aligarhica*. The third worm represents a new Genus for which the name *Periplaneticola mirzaia* is proposed.

A species of Leucophæa has been found infected with a worm belonging to the Genus *Thelastoma* and it has been named *Thelastoma indiana*.

(2) Blattela germanica is infected with a worm which represents a new genus and is named *Blattelicola blattelicola*.

(3) Periplaneta monochroma Walk, Polyphaga ægyptiaca Linn, Supella supellectillum Serv and Phyllodromia humbertiana Saus, were found free of nematode infection.

The writer takes this opportunity to thank Dr. H. S. Pruthi, Imperial Entomologist, New Delhi, for the identification of insects.

Genus *Thelastoma* Leidy, 1849

Synonym.—Bulhœsia Schwenk, 1926.

Generic diagnosis.—*Thelastomatinæ*: Mouth of female surrounded by eight simple sub-median papillæ or sometimes eight labiopapillæ; lateral organs or amphids represented by round or oval openings. Oesophagus consisting of an anterior club-shaped corpus followed by a more or less distinct isthmus and a valvular bulb. Excretory pore anterior to or near base of œsophagus; in certain cases even posterior to it. Tail of female attenuated or filiform, Vulva near middle of body; two ovaries. Eggs without crests or longitudinal grooves. Tail of male elongate, somewhat filiform, bearing four pairs of caudal papillæ, one pair being preanal and ventral in position, and one pair adanal or postanal and subventral in position; in addition there is a postanal projection bearing paired sensory endings, and a pair of papillæ on the tail some distance from the anus.
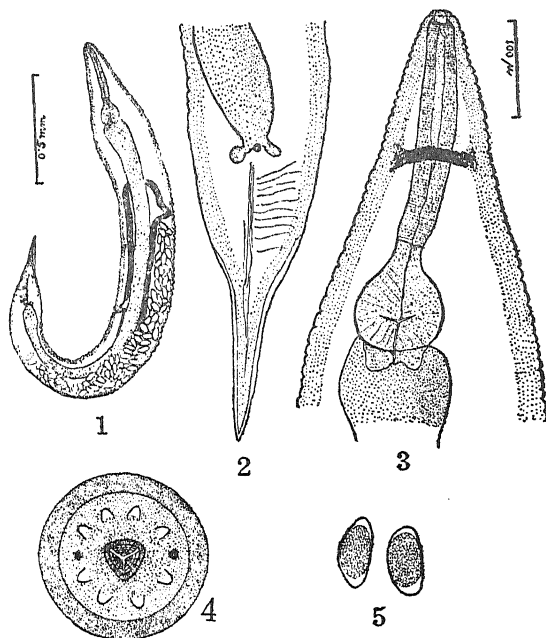
Thelastoma indiana, sp. nov.

FIG. 1. Adult female, lateral view.

„   2. Tail of female, ventral view.

„   3. Female, œsophageal region.

„   4. Female, head, enface view.

„   5. Eggs.

Thelastoma indiana, sp. nov. (Figs. 1 to 5).

Specific description.—Thelastoma:

Male unknown.

Female: 2·48 mm. long by 260 μ wide. Cuticle conspicuously striated throughout the whole length of the body except the tail. First annule 15 μ wide; remaining annules 8 μ to 10 μ wide and more posteriorly 12 μ apart. Oral opening sub-triangular surrounded by eight papillæ. Amphids or lateral organs appear as round openings. Buccal cavity short and wide containing one dorsal and two sub-ventral cuticular elevations. Oesophagus 350 μ long, consisting of a corpus 240 μ long by 40 μ wide, an isthmus 20 μ long by 25 μ wide distinctly set off anteriorly, and a posterior valvular bulb 90 μ long by 90 μ wide. Nerve-ring 150 μ from the anterior end of the body. Excretory pore not observed. Intestine enlarged anteriorly to form a slight

cardia; anus 310 μ from the posterior end of the body. Tail attenuated. Vulva between middle and posterior third of body, 1·48 mm. from the anterior end. Vagina directed anteriorly; uteri divergent. Eggs 75 μ long by 33 μ wide, oval or ellipsoidal, segmented at deposition.

Host.—*Leucophæa* sp. (Family Blattidæ).

Location.—Intestine.

Type Locality.—Aligarh (Northern India).

Type specimen.—Museum of the Zoolog. Labs., Muslim University, Aligarh, Collection No. 1009.
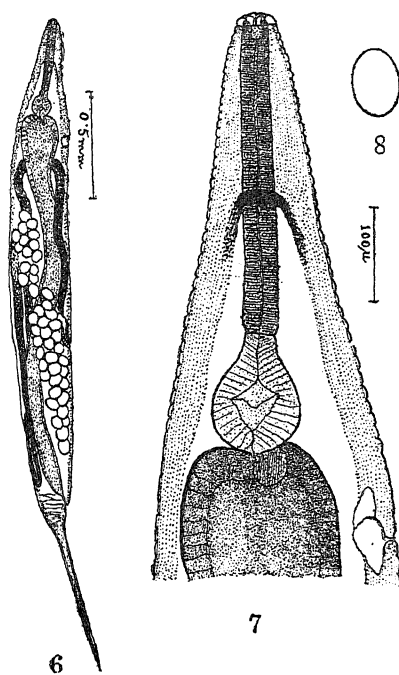
Thelastoma aligarhica, sp. nov.

Fig. 6. Adult female, lateral view.

„ 7. Female, œsophageal region.

„ 8. Egg.

Thelastoma aligarhica, sp. n. (Figs. 6 to 8).

Specific description.— *Thelastoma*:

Male unknown.

Female: 3·3 mm. long by 300 μ wide. Cuticle striated only in the anterior third of the body. Striæ 8 μ apart anteriorly and upto 15 μ apart

posteriorly. Oral opening surrounded by eight labiopapillæ. Amphids present. Buccal cavity 15μ deep by 10μ wide. Oesophagus 460μ long, consisting of a corpus 330μ long by 37μ wide, an isthmus 25μ long by 35μ wide distinctly set off from the corpus, and a posterior valvular bulb 105μ long by 100μ wide. Nerve-ring 185μ from the anterior end of the body. Excretory pore behind the base of the œsophagus, 580μ from the anterior end of the body. Intestine enlarged anteriorly to form a distinct cardia; anus 850μ from the posterior end of the body. Tail filiform. Vulva near middle of body, $1\cdot58$ mm. from the anterior end. Vagina directed anteriorly, amphidelphic. Eggs nearly spherical, 75μ long by 55μ wide.

Host.—Periplaneta americana Linn.

Location.—Intestine.

Type locality.—Aligarh (Northern India).

Type specimen.—Museum of the Zoolog. Labs., Muslim University, Aligarh, Collection No. 1010.

Genus *Blattelicola*, gen. nov.

Generic diagnosis.—Thelastomatinæ:

Male unknown.

Female with œsophagus consisting of an anterior cylindrical corpus followed by a more or less distinct isthmus and a valvular bulb. Tail attenuated. Vulva distinctly posterior about 66 per cent. of the body length from the anterior end, ovaries two. Eggs with two longitudinal lines, segmented at the time of deposition.

Type species.—Blattelicola blattelicola, sp. nov.

The Genus *Blattelicola* appears to be most closely related to the Genus *Severianoia* Schwenk, 1926, Travassos, 1929; but distinctly differs from the latter in the shape of the tail and the position of the vulva. For a detailed comparison the head papillæ could not be observed as there was a single specimen for examination and that too in not a very good condition.
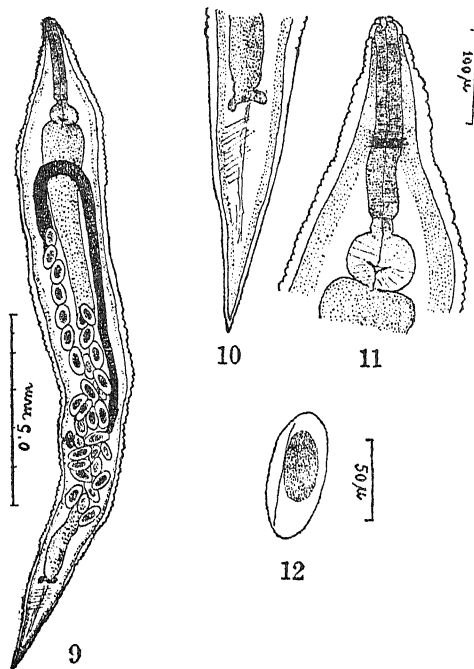
Blattelicola blattelicola, Gen. et. sp. nov.

FIG. 9. Adult female, lateral view.
  „ 10. Tail of female, ventro-lateral view.
  „ 11. Female, œsophageal region.
  „ 12. Egg.

Blattelicola blattelicola, G. et. sp. nov. (Figs. 9 to 12).

Specific description.—Blattelicola :

Male unknown.

Female: 1·75 mm. long by 230 μ wide. Cuticle conspicuously striated. First annule 17 μ wide. Buccal cavity short. Oesophagus 280 μ long, consisting of a corpus 210 μ long by 30 μ wide, an isthmus 15 μ long by 25 μ wide, and a valvular bulb 60 μ long by 75 μ wide. Nerve-ring 125 μ from the anterior end of the body. Excretory pore could not be observed. Intestine enlarged anteriorly to form a distinct cardia. Anus 250 μ from the posterior end of the body. Uteri divergent. Eggs ellipsoidal 80 μ long by 35 μ wide, bearing two sinuous longitudinal lines; deposited in morula stage.

Host.—Blattela germanica Linn.

Location.—Intestine.

Type locality.—Aligarh (Northern India).

Type specimen.—Museum of the Zoolog. Labs., Muslim University, Aligarh, Collection No. 1011.

Genus *Periplaneticola*, gen. nov.

Generic diagnosis.—*Thelastomatinæ*:

Mouth opening surrounded by eight labiopapillæ. Oesophagus consisting of an anterior corpus followed by a distinct isthmus and a valvular bulb. Tail of female very characteristic, short and blunt with a very small caudal appendage distinctly set off from the body. Vulva distinctly posterior, about 66 per cent. of the body length from the anterior end. Uteri divergent. Eggs not observed.
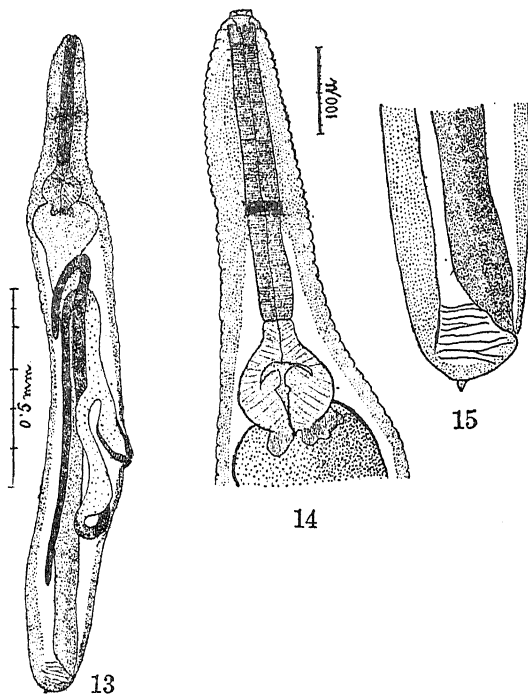
Periplaneticola mirzaia, Gen. et. sp. nov.

FIG. 13.   Nongravid female, lateral view.
   ,,   14.   Female, œsophageal region.
   ,,   15.   Female, Tail, lateral view.

Periplaneticola mirzaia, sp. nov. (Figs. 13 to 15).

Specific description.—Periplaneticola:

Male unknown.

Female (nongravid): 1·67 mm. long by 200 μ wide. Cuticle striated nearly throughout the whole length of the body. Posterior striations not very conspicuous. First annule 15 μ wide, remaining annules 10 to 13 μ apart. Oral opening surrounded by eight labiopapillæ. Buccal cavity cylindrical and sufficiently wide, 18 μ in depth, containing one dorsal and two subventral anteriorly projecting somewhat conical cuticular structures. Oesophagus 440 μ long, consisting of a corpus 330 μ long by 35 μ wide, an isthmus 20 μ long by 35 μ wide, and a valvular bulb 90 μ long by 100 μ wide. Nervering 215 μ from the anterior end of the body. Excretory pore not observed. Intestine enlarged anteriorly to form a cardia. Anus 80 μ from the posterior end of the body. Tail short and blunt with a very small caudal appendage distinctly set off from the body. Vulva 1·1 mm. from the anterior extremity. Uteri divergent. Ovaries two. Eggs not observed.

Host.—Periplaneta americana.

*Location.—*Intestine.

*Type locality.—*Aligarh (Northern India).

*Type specimen.—*Museum of the Zoolog. Labs., Muslim University, Aligarh, Collection No. 1012.

Genus *Hammerschmidtiella* Chitwood, 1932

Species *Hammerschmidtiella diesingi* (Hammerschmidt, 1838).

Synonyms.—Oxyuris diesingi Hammerschmidt, 1838 ;

Oxyuris blattæ orientalis Hammerschmidt, 1847 ;

Streptostomum gracile Leidy, 1850 ;

Anguillula mercrura Diesing, 1851 ;

Aorurus diesingi Hammerschmidt, 1838, Travassos, 1929.

Host.—Periplaneta americana.

*Location.—*Intestine.

*Locality.—*Aligarh (Northern India).

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A REPORT ON THE CHARACTERS AND IDENTIFICATION OF THE YEASTS LIVING IN COMMENSALISM IN THE INTESTINE OF SOME LABORATORY ANIMALS

BY PROF. COL. I. FROILANO DE MELLO

IN the present study we will define the characters of and try to classify the yeasts isolated from the intestine of some laboratory animals submitted to experimental beberigenic diet, the results of which have been reported in a previous paper.[1]

1. *Characters of the yeasts isolated from the stools of white rats* (in collaboration with my pupil Jonas de Sã Viegas).

N = Control animal; E = animal under experiment.

Technique.—Primary culture in bouillon with 4% glucose + 1% yeast beer. Isolation in Petri dishes with glucosed and maltosed Sabouraud. Sub-cultures in:

Solid Media

	Carrot	Glycerinated carrot	Potato	Glycer. potato	Sab. malt.	Sab. gluc.
E 1	abundant, white, waxy	imperceptible	abundant, grey whitish	whitish waxy, later on chalky	white waxy, abundant	..
E 2	id., a little more dry	id.	poor, waxy with shining reflexes	id.	id.	..
E 3	id, wrinkled	id.	greyish white, dry, wrinkled	id.	id.	..
E 4	ut E 2	regular, white waxy	id., waxy with shining reflexes	waxy	id.	..
N 1	waxy white with shining reflexes	wet, yellowish	whitish, in small dots	white papulæ-like colonies	id.	..
N 2	white waxy in small dots	ut in carrot	id.	imperceptible	whitish col. in small dots the confluence of which gives later on a verrucose membrane light brown	ut Sab. malt
N 3	ut N 1	wet, small dots shining	wet, whitish, shining	whitish	ut N 1	id.
N 4	id.	id.	verrucose, whitish	very weak develop	ut N 2, the membrane harder and more pigmented. Rapid growth of R. forms	id.
N 5	id.	develop. whitish, membraniform	whitish, waxy	whitish, membraniform	ut N 1	id.

N.B.—Cultures on slices of citron and cooked rice grains are of no value macroscopically; they give, however, very good elements for mycological identification.

Liquid Media

	Milk	Vegetable bouillon	Rice water	Carrot bouillon	Bouillon	Langeron's milieu
E 1	Partial coagulation	Veil. Depot at the bottom, Milieu darkened	Veil. Depot	Veil. Depot	Trouble	Veil. Depot
E 2	id.	id.	id.	id.	Depot, clear	id.
E 3	id.	id.	id.	id.	Veil. Depot	id.
E 4	id.	id.	id.	id.	Membraneous depot	id.
N 1	id.	Depot half membraneous, half sandy	id.	id.	ut E 2	id.
N 2	nihil	Veil. Depot	hardly visible	id., veil very developed	id., veil very develop	id., Depot small
N 3	id.	ut E 1	id.	id.	id.	id.
N 4	Coagulation	ut N 2	id.	verrucose veil	verrucose veil	Veil. Depot
N 5	Partial coagulation	ut E 1	ut E 1	ut N 1	ut N 2	ut N 2

Action on Sugars.—The sugars used were: glucose, maltose, levulose, galactose, mannite, inuline, xylose, dextrine, lactose. All remained unaltered, excepting those recorded below:

	Gluc.	Malt.
E 1	AG	AG
E 2	O	A
E 3	AG	AG
E 4	O	AG
N 1	O	O
N 2	O	A
N 3	O	O
N 4	O	O
N 5	O	O

Form and Measurements of the Yeast Cells

	Form	Measurements in microns
E 1	Round, oval	R 3; 5. Ov 2, 5/1; 3/1
E 2	oval	Ov 4/3; 5/2
E 3	id.	Ov 5/2; 5/3
E 4	id.	Ov 3/1; 4/2; 5/3
N 1	Round, oval	R 2; 4. Ov 3/1; 5/2
N 2	oval	Ov 2/1; 4/2; 5/3
N 3	id.	Ov 2/1; 3/1; 4/2
N 4	id.	Ov 2/1; 4/2; 5/3
N 5	id.	Ov 3/2; 5/2; 6/3; 6/4

Mycological Elements enabling the Generic Classifications of the Strains—

The strains above reported may be reduced, apart small individual variations, to two main groups:

(*a*) The first one includes all the strains labelled E and those labelled N 1, N 3, N 5. Their micromycology, studied in different media shows yeast like cells, oval or roundish, in binary gemmation or with crown lateral

multiple budding; mycelial filaments, more or less long, according to the milieu, septuated, ramified, with granular protoplasm; lateral or terminal clusters of conidia, some of them being attached to daughter cells in a form of chains; 8 like cells; metachromatic grains in yeast cells. Intermediate chlamydospores. No asci.

These elements enable me to classify all these strains in the genus *Candida* Berkhout.

(*b*) The strains N 2 and N 4, moreover their totally different macroscopic aspect, show in micromycology some new elements, worthy of remark. In N 2, even in old cultures there are arthrospores and blastospores. In N 4, in old cultures there are the same elements and, at the terminal end of the hyphæ, some small roundish cells which seem to be also arthrospores. In young cultures there are many chlamydospores and terminal blastospores. It seems that these elements give rise in old cultures to short mycelial filaments having the form of blastarthrospores. No asci.

The elements recorded above enable me to classify these two strains in the genus *Geotrichoides* Langeron and Talice.

Specific Identification.—The characters of these strains on different media should be interpreted, at present, as only individual variations. We do not consider them as different species and we will not push forward the identification of these strains. The actual position of the anascosporated yeasts is in such a confusion that we prefer to send all our specimens to the Central Bureau in Baarn (Holland) for further studies and comparison with type cultures.

Inoculation.—Inoculated intramuscularly in pigeon, intraperitoneally in white rats, intraveinously in rabbits—results all negative.

Summarising the statements:

The yeast living in the intestine of white rats in a state of normal commensalism and devoid of any pathogenic power for white rats, pigeons and rabbits, belong to the genera *Candida* Berkhout and *Geotrichoides* Langeron and Talice.

2. *Characters of the yeasts isolated from the stools of the hens* (in collaboration with my pupil Armando Baptista Cardoso).

N = control animal; E = animal under experiment.

Technique.—The same as described in page 17.

Solid Media

	Carrot	Glycer. carrot	Potato	Glycer. potato	Sab. malt	Sab. gluc.	Plain agar
N	Dry, membraneous, powdery surface	imperceptible	white creamy	imperceptible	white creamy	ut Sab. malt	weak development in small colonies
E 1	id.	id.	white, membraneous	id.	id.	id.	id.
E 2	id.	id.	id., less develop.	id.	id.	id.	id.
E 3	id., the membrane frankly chalky and folded, becoming later on verrucose and brownish	membraneous invading the walls of the culture tubes	ut on carrot	verrucose, partially chalky, becoming brownish	white powdery, becoming later on yellow brown, verrucose	id.	ut Sab. malt
E 4	ut E 3, but at first in small dots	imperceptible	id.	imperceptible	ut E 3	id.	id
E 5	ut N	regular development	creamy whitish	weak development	ut N, a little more wet	id.	ut N

N.B.—*Culture on citron slices*: Small, rather imperceptible cultures, except for E 3 where the development is abundant, chalky, invading the walls of the tube.

Liquid Media

	Carrot bouillon	Langeron	Rice water	Vegetable bouillon	Plain bouillon	Milk
N	Veil. liquid clear	Veil. flocky depot	Veil. Rudimentary depot	Veil. Depot	slight depot	nil.
E 1	id.	id.	id.	id.	Veil, dep.	id.
E 2	Rudimentary veil, depot	Veil. Rudimentary depot	id.	Veil. Rudimentary depot	Veil. Rudimentary depot	id.
E 3	Veil. Depot	Veil. Depot	Veil. Depot	Veil. Depot	Veil. Depot	coagulation peptonisation
E 4	id.	id.	id.	id.	id.	coagulation
E 5	id.	id.	id.	id.	id.	nil

Action on Sugars.—Lactose, dextrine, arabinose, mannite, inuline—nihil.

	Saccharose	Glucose	Maltose
N	A	AG	A
E 1	A	O	O
E 2	O	O	O
E 3	O	O	O
E 4	O	O	O
E 5	A	AG	A

Experimental Inoculations.—Inoculated intraveinously in rabbits, intramuscularly in pigeons, intraperitoneally in white rats—Results all negative.

Form and Measurements of the Yeast cells

		On Carrot	On potato
N	Oval Round	5/2; 5/3; 6/2; 7, 5/3 2, 5	Ov 3/2; 3/2, 5 R 2; 2, 5; 3
E 1	Ov R	2, 5/2; 3/1; 4/2; 4/2, 5 2	
E 2	Ov R	2, 5/2; 4/2, 5; 5/2; 6/3 2	
E 3	Ov R	2, 5/2; 3/2, 5; 5/2; 6/2, 5 2, 5	
E 4	Ov R	4/2; 5/2, 5; 8, 5/2, 5 2; 2, 5	
E 5	Ov	2, 5/1; 3/2; 4/2; 5/2, 5	Ov 4/3; 5/2, 5; 5/3; 6/3 R 6

Mycological Elements enabling the Generic Classification of the Strains.—

The mycological elements observed in all these strains enable us to classify them as belonging undoubtedly to the genus *Candida* Berkhout. We are dealing with two different types:

(*a*) The one constituted by the strains, N, E 1, E 2 and E 5.

(*b*) The second, by the strains E 3 and E 4.

The differential characters between them are that the type (*a*) shows a white creamy culture which becomes afterwards waxy and the type (*b*) cultures which are at first white, somewhat powdery, becoming afterward verrucose and brownish.

3. *Characters of the yeasts isolated from the stools of rabbits* (in collaboration with my pupil Miguel A da Costa).

N = control animal; E = animal under experimental regime.

Technique.—The same as above.

Solid Media

	Carrot	Glycer. carrot	Potato	Glycer. potato	Sab. gluc.	Sab. malt
N	Wet, smooth, some-what shining	ut in carrot	creamy	imperceptible	white, mother of pearl colour	ut Sab. gluc.
E 1	White	id.	wet, yellowish	weak	white, smooth shining	id.
E 2	White, under the form of a thin membrane	imperceptible	weak develop., yellowish	whitish, rather dry	white, membraniform	id., strongly develop.
E 3	Thin membrane, somewhat shining	smooth, whitish shining surface	yellowish	imperceptible	Smooth shining	ut Sab. gluc.
E 4	Creamy, yellowish	whitish, weak develop	waxy	id.	creamy	id.

N.B.—*Culture on citron slices.*—N, E 1, E 3 imperceptible; E 2 abundant, whitish; E 4 membraniform, whitish.

Liquid Media

	Plain bouillon	Carrot bouillon	Vegetable bouillon	Langeron's milieu	Rice water	Milk
N	Clear, depot	Veil. Depot	Veil	Veil. Depot	nihil	?
E 1	id.	id.	Veil. Depot	clear, Depot	..	Peptonised
E 2	Veil. Depot	nihil	id.	id., veil	nihil	id.
E 3	id.	veil	id., clear	Veil. Depot	?	nihil
E 4	i.l.	Depot	clear-veil	id.	?	i.l.

Action on Sugars.—(Glucose, lactose, maltose, xylose, inuline, dextrine, arabinose). AG in glucose in N strain. No other sugar fermented with any strain.

Form and Measurements of the Yeast Cells

N	Oval 1/0, 5; 2/1; 3/1, 5; 4/1, 5; 4/1, 5; 4/2
E 1	Ov 3/1; 3/2; 5/1
E 2	Ov 1/1; 2/1; 3/2; 4/1, 5; 4/2; 5/2
E 3	Ov 3/1; 3/1, 5
E 4	Ov 3/2; 4/2

Experimental Inoculations.—Rabbit (intraveinous inoculations), pigeon (intramuscular), white rat (intraperitoneal)—Negative results.

Mycological Elements enabling a Generic Classification.—All the strains show the same type of mycological elements: yeast like cells with binary or multiple budding; lateral and terminal clusters of conidia; irregular coraliform elements; pseudo-myceliums; long mycelial threads, septuated, ramified, granular, sometimes recurved. In E 1 there are also very thin mycelial threads resembling Nocardia. No asci. The fructification belongs to the genus *Candida* Berkhout, from which we have here two types: (*a*) the one constituted by the strain N; (*b*) the second constituted by the strains E 1, E 2, E 3 with a slight variation in E 4.

4. *Characters of the yeasts isolated from the stools of the Pigeons* (in collaboration with my pupil Luis Sales d'Andrade e Souza).

N = Control animals; E = animals under experimental regime.

Technique.—The same as above.

Solid Media

	Potato	Glycer. potato	Carrot	Glycer. carrot	Sab. malt	Sab. gluc.	Plain agar
N 1	white waxy	imperceptible	ut on potato	imperceptible	whitish creamy	ut Sab. malt	whitish creamy, weak develop.
N 2	id.	di.	id.	id.	id.	id.	id.
N 3	smooth, shining	id.	id.	slight develop.	smooth, shining, brownish	id., brownish tone less pronounced	small colonies, brownish
E 1	white waxy	id.	id.	imperceptible	whitish, creamy	ut Sab. malt	nihil
E 2	id.	slight membrane	id.	id.	id., but developing in colonies of various sizes	id.	ut N 1
E 3	id.	imperceptible	id.	id.	ut N 1	id.	nihil
E 4	id.	id.	id.	id.	id., somewhat yellowish	id.	ut N 1

N.B.—Examined 60 days after all cultures show a slight brown grey pigmentation. The strain N 3 shows its surface finely mamelonated.

Liquid Media

	Carrot bouillon	Vegetable bouillon	Bouillon	Langeron's milieu	Milk
N 1	slight veil, depot	slight veil, depot clear	slight veil, depot	Veil, depot	nihil
N 2	id.	id.	id.	id.	id.
N 3	id.	id., milieu deep brownish	id.	?	peptonised
E 1	id.	Veil, depot, clear	id.	ut N 1	nihil
E 2	id.	id.	id.	id.	id.
E 3	id.	id.	id.	id.	id.
E 4	id.	id.	id.	id.	id.

N.B.—*Culture on citron slices*: N, E 1, E 3 imperceptible; E 2 abundant, whitish; E 4 membraniform, whitish.

Action on Sugars.—(Glucose, lactose, arabinose, maltose, dextrose, mannite, inuline, saccharose)—nihil.

Form and Measurements of the yeast cells

N 1	Round 2; 3;	Oval 3/2; 4/2; 5/3
N 2	R	1, 5; 2, 5	Ov 3/2; 1, 5/1; 5/2, 5; 5/3
N 3	R	1, 5; 2, 5	Ov 3/1; 2, 5/1; 1, 5/1
E 1	R	1	Ov 1, 5/1; 3/2; 4/2
E 2	R	3	Ov 6/3; 3/2; 5/3
E 3	R	2	Ov 5/3; 6/3; 4/3
E 4	R	2	Ov 3/2; 4/2; 5/2; 5/3

Inoculations to animals.—Rabbit (intraveinous), Pigeon (intramuscular), white rat (intraperitoneal).   Negative.

Mycological Elements enabling a Generic Classification.—

We are in the presence of two kinds of yeasts:

(*a*) The first one, constituted by the strains N 1, N 2, E 1, E 2, E 3, E 4, apart small individual variations, belong all to the same genus, *Candida* Berkhout.

(*b*) The strain N 3 is entirely different. Macroscopically, pigmented since the beginning, it shows the following mycological elements: yeast cells, often with irregular borders, sometimes grouped in coraliform elements.   Blastospores, blastarthrospores and chlamydospores of varied sizes. Rare filaments of *Nocardia* type with protoplasmic condensations resembling to spores (pseudospores), some of these filaments being recurved or fusiform.   Short navicular mycelial filaments, often assembled in pairs giving thus the aspect of a binary division.

We classify the strain N 3 as a *Geotrichoides* Langeron and Talice, Cifferi and Redaeli.

Characteristics and Affinities of the Genus Geotrichoides Longeron and Talice 1932.[2]

Membrane forming colonies, developing in thick layer, flat, plicate or areolate, often with powdery surface (duvet).   Blastarthrospores oval or pyriform, in crescent form, or ondulated and giant.   Pseudomycelium extremely polymorphous, with slightly ramified filaments, difficult to be

dissociated and filled up with oil droplets. The wall of the articles and of blastarthrospores has often a double contour. Coremia more abundant in membranaceous cultures. Verticils with irregular and not numerous blasto-spores. There are no true chains of arthrospores.

Cifferi and Redaeli have made some alterations in the above diagnosis, considering *Geotrichoides* as a sub-genus of *Trichosporum*, *Behrend*. So, *Geotrichoides* (Lang. and Tal.) Cif. and Red. *nov. comb.*, has the following characters[3]:

Membrane forming colonies, thick, plicate or areolate, smooth or pow-dery or with dendritic filaments; multiplication through blastarthrospores of various forms, often through blastospores. True arthrospores missing. Mycelium highly developed, polymorphous and often formed by elongated cells which may be isolated.

As said above, these Italian authors consider *Geotrichoides* as well as *Proteomyces* (Moses and Viana, 1912) Cif. and Red. *nov. comb.*, as subgenera of *Trichosporum* (the designation *Proteomyces* is given by Brumpt as synonym of *Geotrichum* Link, 1809).

To the same genus *Geotrichoides* belongs the yeast, agent of a case of onychomycosis which I wrongly classified as a *Torulopsis* Berlese.[4]

Anyhow, owing to constant changes in the classification of these anascos-porated yeasts all our strains have been forwarded to the Central Bureau var Schimmelcultures to Baarn, Holland, for their ulterior identification.

Conclusion

The yeasts which are normal commensals in the intestine of white rats, hens, rabbits and pigeons belong to the genera *Candida* Berkhout and *Geo-trichoides* (Langeron and Talice) Cif. and Red.

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HIS LATE HIGHNESS MAHARAJA
SRI KRISHNARAJA WADIYAR BAHADUR, G.C.S.I., G.B.E.
MYSORE
(4TH JUNE 1884 — 3RD AUGUST 1940)

HIS LATE HIGHNESS MAHARAJA
SRI KRISHNARAJA WADIYAR BAHADUR
G.C.S.I., G.B.E.

(4th June 1884—3rd August 1940)

THE sudden death of His Highness Sir Sri Krishnaraja Wadiyar Bahadur, the late Maharaja of Mysore, has come to us as a rude shock, and leaves his subjects with a sense of acute personal loss.

It is impossible in the short space at our disposal to give an adequate account of his long and illustrious reign in the course of which he built for Mysore a reputation abroad of being the most progressive State in India. His great personal qualities endeared him to all those who came in contact with him. His simplicity of life, religious feeling, humanitarian outlook, great sense of duty and above all his extreme devotion to the welfare of his subjects were known by repute to people throughout India who had no connection with the State of Mysore. He will always be cited as an example of what a great Ruler should be.

But posterity will perhaps remember him above all for having begun and carried through to a great extent the Industrialization of the State of Mysore. He was a great patron of the Arts and Sciences. To him is largely due the establishment of the Indian Institute of Science in Bangalore, made possible by the generous grant of a vast extent of land and a handsome gift of money supplemented by an annual contribution. He laid the foundation stone of the Institute in the year 1911. The Indian Academy of Sciences, in particular, owes a deep debt of gratitude to His Highness and to the equally far-sighted Dewan, Sir Mirza M. Ismail, for a generous annual grant to the Academy and the gift of a large extent of land in a commanding situation in Bangalore for the

purpose of building a permanent home for the Academy. There is no space to enumerate the very material patronage given by him to every Scientific and Educational Institution, Hospitals, Agricultural Projects and Rural Development Centres.

His Highness's love of the Arts, in particular, Music, is equally well known. Through it was made possible the enlightened policy of the present Dewan of beautifying the State of Mysore by the making of large parks, and the construction of handsome public buildings so that the subjects of Mysore and especially the rising generation may develop in them a sense of beauty, orderliness and pride in their State such as is found in Europe.

As President of the Indian Academy, I most respectfully beg to convey the condolences of the Fellows of the Indian Academy of Sciences to Her Highness the Dowager Maharani of Mysore, to His Highness Sri Jayachamaraja Wadiyar and the other members of the Royal Family for the sad bereavement they have sustained.

C. V. RAMAN.

STUDIES IN THE SOUTH INDIAN CHILLIES

I. A Description of the Varieties, Chromosome Numbers and the Cytology of some X-rayed Derivatives in *Capsicum annuum* Linn.

BY PROFESSOR T. S. RAGHAVAN, M.A., PH.D. (Lond.), F.L.S.

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CONTENTS

I. *Introduction*

THE cultivation of the genus *Capsicum* is more or less widespread in South India. Its home is said to be South America, growing wild on the banks of the Amazon and in Eastern Peru. Its exotic nature could be gathered not

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only by its not being mentioned in ancient Sanskrit literature but also by the fact that for religious Hindu rituals, *Capsicum* is not used for culinary purposes.

The earlier workers like Roxburgh (1832) recognised a large number of species of Indian Chillies. But Irish (1898) reduced the number of species to two—*Capsicum annuum* Linn. and *Capsicum frutescens* Linn. These are divided into a number of varieties. The species of Roxburgh, like *C. purpureum*, *C. grossum*, etc., and those of earlier writers, come under *C. annuum* as varieties.

Not much work seems to have been done on the Indian Chillies. Shaw and Abdur Rahman Khan (1928) studied 52 types of Indian Chillies. Of these the bulk belonged to Northern India, only a few were South Indian varieties. Dixit (1933) described mitosis in *Capsicum*. He has, however, paid no attention to the morphology of the chromosomes.

Shaw and Khan recognised only two types of *C. frutescens*, whilet he rest were varieties of *C. annuum*. In the present study, an attempt has been made to collect the various types of South Indian Chillies. By the kindness of the Director of Agriculture, Madras, seeds from various parts of the Presidency were got, and grown in the University Botanic Gardens; a few were got from Pochas, Poona.

In the classification of the South Indian varieties, only such characters have been employed as have already been utilized and wherever possible these varieties have been assigned to type forms already described by Shaw and Rahman Khan. This is adopted with a view to avoid multiplication of types. And varieties not coming within those already described are specified by their localities. Also very minor characters have not been employed as this would also lead to a multiplication of the types. Table I gives an account of the data collected from the various localities, in respect of the time of sowing, transplantation and the first and last pickings.

TABLE I

Varieties	Sowing	Transplanting	1st picking	Last picking
1. Chodavaram	August	October	January	April
2. Erode	June	August	November	March
3. Chandragiri ..	January	March	..	..
4. Cheparupalli	July September	August December	December	March
5. Tiruttani	January	..	..	..
6. Nandyal	July	August	November	
7. Allagadda	July	..	..	..
8. Madura	August	September	December	March
9. Paramakkudy	September	..	..	April
10. Sattur	August	..	December	March
11. Periyakulam	February	March	..	..
12. Musiri	August	October	January	April
13. Kulittalai	August	October	January	April
14. Tanjore	August	September	..	February
15. Atmakur	September	October	January	April
16. Kandukur	August	..	January	April
17. Kaveli	November	December	March	April
18. Nellore	October	November	March	..
19. Gudiyattam	December	Feburary	May	July
20. Walajah	June	August	..	..

III. *Short Description of Some Types of South Indian* Capsicum

All the varieties belong to *Capsicum annuum* (flowers solitary). In all the varieties the flowers are white.

Kadiri 1.   Plants are $1\frac{1}{2}$ to 2 feet high; not bushy. Fruits are thin and long (3 to $3\frac{1}{2}''$), pendent, red wrinkled; calyx embracing the base of the fruit; similar to type 44 of Shaw and Rahman Khan.

Thiruvannamalai, Guntur 398 and *Bellary* varieties are like the above.

Kadiri 2. Plants slightly bigger; fruits medium sized 2 to $2\frac{1}{2}''$ long, conical, red pendent, smooth surface; calyx embracing; similar to type 36.

Cheparupalli varietiy is similar to above.

Gudyattam like Kadiri 2 but fruits shorter; about 1″ long.

Nandyal like Gudyattam.

Perambalur 2 like Gudiyattam.

Paramakkudy plants about 2 feet; fruits globular large, red smooth surface; calyx not embracing.

Cape medium 2 like Paramakkudy but the fruits are orange coloured.

Pithapuram. Plants 2 feet high; fruits are long (3 to 3½″), smooth orange coloured, circular in transverse section; calyx not embracing; similar to type 13.

Sunnybrook. Leaves bigger than the rest as also the flowers. Fruits large, globular, angular in cross-section, corrugated base, pendent.

Californian Wonder. Similar to above but with erect fruits.

Atmakur. Small, globular, circular in cross-section, pendent, orange colour; calyx not embracing.

Kulittalai 2 and *Kandukkur* are similar to above, but the fruits red.

Chodavaram. Similar to above.

Sattur. Plants about 4 feet high; leaves light yellow; fruit 1½ to 2″ long, red not wrinkled; calyx embracing; like type 38.

Thiruthony and *Thirumangalam* are similar to Sattur.

Local (Chidambaram). Plants about 2 feet high; fruit 1½ to 2″ long, red, pendent; calyx not embracing.

Kaveli, *Kulittalai* 1, *Cape* 1 and *Puthoor* are similar to the local variety.

Tanjore. Fruits are globular, large, completely corrugated, pendent red and calyx not embracing.

Travancore. Fruits short about an inch long, somewhat blunt, red; calyx not embracing.

Musiri and *Walajah* 1 are similar to Travancore.

Guntur 380. Fruits short, pendent about an inch long, orange coloured and calyx embracing.

Nellore. Fruits about 2″ long, orange and calyx embracing.

Perambalur 1. Fruits long, 2 to 2½″, red, acute tip; calyx embracing.

Kovoor and *Nayadupet* varieties are just like Perambalur 1.

Chandragiri. Short conical about an inch, pendent, orange and calyx not embracing.

Allagadda. Long, 2 to 2½″, wrinkled, angular and calyx not embracing.

Cuddalore. Fruits medium-sized, 1 to 1½″ long, orange; calyx not embracing. *Walajah 2 is similar* to above.

IV. Classification of the Varieties

Capsicum annuum (Flowers solitary)

FLOWERS white

Fruits globular — Fruits elongated

Fruits globular:

Large and angular — Small and circular

Large and angular:
- Smooth surface
- Completely corrugated (Tanjore)
- Corrugated at base

Smooth surface:
- Orange — Cape Med. II
- Red (Paramakkudy)

Corrugated at base:
- Pendent (Sunnybrook)
- Erect (Cal. Wonder)

Small and circular:
- Pendent
  - Orange (Atmakur)
  - Red (Kulittalai) (Kandukur) (Chidavaram)
- Erect

Fruits elongated:

Calyx not embracing — Calyx embracing

Calyx not embracing:
- Erect
- Pendent
  - Circular
  - Angular in T. S. (Allagadda)

Circular:
- Orange
  - Long (Pithapuram)
  - Medium (Cuddalore) (Walajah 2)
  - Short (Chandragiri)

Angular in T. S. (Allagadda):
- Red
  - Long (Kulittalai I) (Kaveli) (Local) (Puthur) (Cape I)
  - Short (Travancore II) (Musiri) (Walajah 1)

Calyx embracing:
- Pendent — Erect

Pendent:
- Orange
  - Short (Guntur 380)
  - Long (Nellore)
- Red
  - Short (Gudiyattam 30) (Nandyal) (Perambalur 2)
  - Medium (Kadiri 2) (Cheparupalli)
  - Long

Long:
- Blunt
  - Wrinkled (Kadiri 1) (Guntur 398) (Tiruvannamalai)
  - Not wrinkled (Tiruttani) (Sattur 38) (Tirumangalam)
- Acute tip
  - (Perambalur 1) (Kovvur) (Nayudupet)

V.　X-ray Treatment and Origin of Material

Seeds of a pure line of Paramakkudi were exposed to X-rays. They were treated in a dry state and the treatment consisted of unscreened exposure under a water-cooled Coolidge tube in the copper anticathode operated at 53 kv and a tube current of a 10 m. A., at a target distance of 17 cms. for one hour. We are indebted to Capt. T. W. Barnard for this.

Several types of mutations affecting size of plant, branching, chlorophyll content of leaves, size of leaves, nature of fruit—pendent or erect—, size of the fruit were noticed in the X–1 generation. Of these six have been isolated for further study. They are shown in the accompanying photographs. They are *Pa* X–1 "*large-leaved giant*" which is twice the size of the normal plant, bushy and leaves very large (7" × 4"). Fruit pendent and normal (Pl. I, Fig. 4).

Pa X–2 "*narrow-leaved giant*". Similar to *Pa* X–1 but the leaves are very narrow. Fruit pendent and normal (P. I, Fig. 4).

Pa X–3 "*lean erect*" plants are very lean about 20 inches high and a spread of 9". There is a conspicuous absence of branches from the base. The fruits are erect, of normal size rounded and arising singly (Pl. I, Fig. 3).

Pa X–4 "*bushy erect*". The plant was nearly 2 feet high and a foot across; branching profuse from the base. Fruits erect (Pl. 1, Fig. 1).

Pa X–5 "*nigroides*". Short spreading, dark green leaves like those oᵢ *Solanum nigrum* (Pl. I, Fig. 5).

Pa X–6 "*dwarf*". Short, no branches at the base (Pl. I, Fig. 2).

The bulk of the cytological data herein presented relate to those of a 'semi-sterile mutant of the X–2 generation' and of a normal looking plant of the X–1 generation.

Anthers were fixed in Navashin after acetocarmine examination and sections were made in the usual way using Genetian violet as the stain.

VI.　Observations

(*a*) *Somatic Chromosomes.*—The somatic chromosome number in all the varieites, is 24. The complement in two such widely different varieties as Paramakkudi and "Sunnybrook" are analysed and it will be seen that the range of size is small in both cases (Text-Figs. 1–4). The cetromeres are of the median and sub-median type. There are three pairs of long chromosomes in both. In Paramakkudi one of these has median constriction while in "Sunnybrook" two have it. In the other chromosomes there is slight
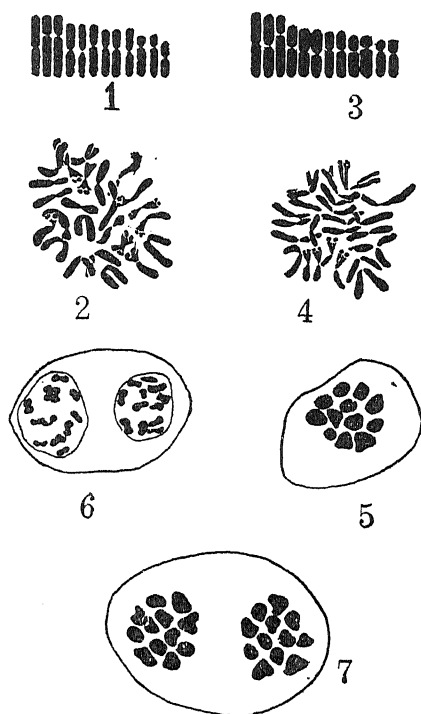
variation in the position of the centromere. It is obvious that change in chromosome number has not played any part in the form of the varieites, however different they are, and one has to look to structural changes rather than number of even morphology of chromosomes.

Meiosis of the un-X-rayed plant is normal and this was found to be true in half a dozen varieties. Fig. 5 shows M I with 12 bivalents and Fig. 7 shows M II plate with 12/12 arrangement. Pollen is formed in the normal manner. Fig. 6 shows interphase nuclei with 12 univalents in each.

(b) *Asynapsis.*—This was very common in a semi-sterile mutant of the X–2 generation. In many cases, the full diploid number of chromosomes is seen at diakinesis (Fig. 8). This could arise from a restitution nucleus formed at first division including all the 24 chromosomes. This, however, is improbable because, restitution nuclei are usually irregular in shape and do not generally include all the chromosomes. This appears to be a case of complete failure of pairing. This asynapsis has been frequently recorded as a result of X-rayed mutation. Goodspeed (1929) has described a first metaphase with 48 univalents in place of 24 bivalents. In Fig. 9, there is a tendency for bivalent formation, there being a single ring bivalent and the rest are univalents.

The occurrence of full diploid number of univalents without any pairing whatsoever, is common in interspecific hybrids. In *Narcissus*, Nagao (1933) found no pairing of parental chromosomes at all. Partial or absence of pairing in hybrids is regarded as indicating distant relationship of parental species. The chromosomes of the parental species are considered to be structurally dissimilar and therefore incapable of pairing at meiosis. Though this general principle was found to be true in *Nicotiana* (Goodspeed, 1934), *Crepis* (Babcock and Emsweller, 1936), etc., there are a number of other cases where this is not revealed. Crew and Koller (1935) consider this abnormality along with others to be genotypic rather than structural. Sapehin (1933) opines that if the genotype is kept constant other changes like the change of environment may induce failure of conjugation of chromosomes
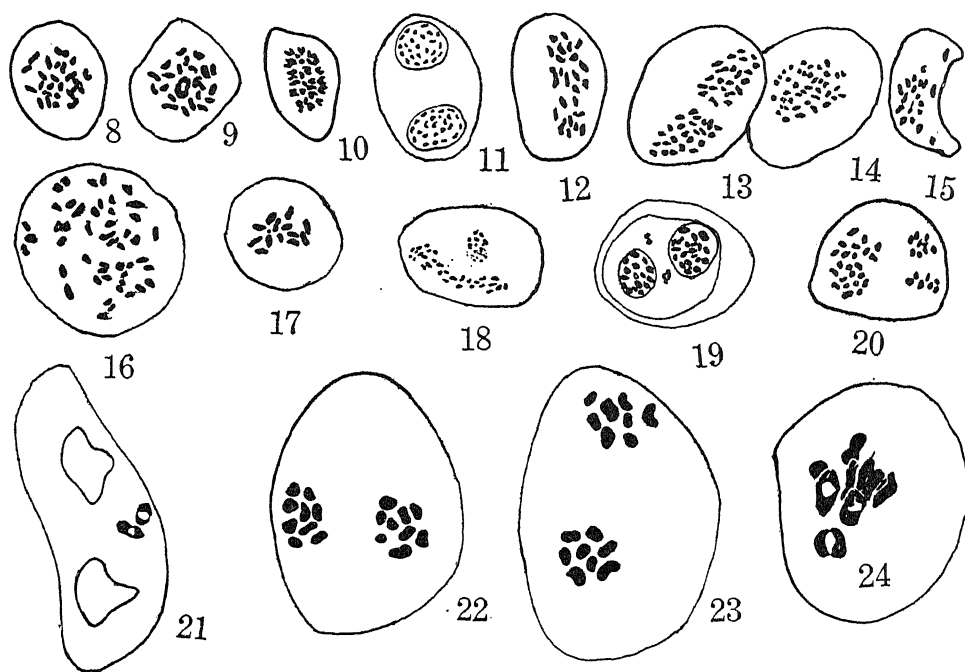
as the response of a particular genotype to the new condition. To this category according to him must belong the abnormalities in meoisis induced by external agencies like temperature X-rays, etc. In *Capsicum* there is evidence of structural changes, however slight, having been induced by X-rays. The not infrequent occurrence of chromosome rings of four, is a sure indication of this. Asynapsis has been recorded in a number of other plants in some of which it has been traced to the presence of recessive genes. Since this paper

was being prepared for the press we have seen a note by Pal and Ramanujam (1940) describing the occurrence of asynaptic chilly in a natural population.

The behaviour of the unpaired univalents is varied. Sometimes no further division takes place and a membrane is organised and presumably a monad with 2 *n* chromosome is formed (Fig. 10). Frequently, these univalents assort themselves at random (Fig. 12) and nuclei with irregular chromosome numbers are found. Fig. 46 is a cell with such irregularly formed nuclei. Sometimes a cross-wall is formed separating these and the nuclei of different sizes are seen in the two cells (Fig. 48). Fig. 18 shows an earlier stage where these univalents are in a process of assortment into groups prior to organisation into irregular nuclei. Fig. 20 shows another type of irregularity. The prevailing method seems to be, however, for these univalents to undergo mitotic division. Fig. 16 shows nearly 48 bodies and these appear to have been derived by the splitting of each univalent prior to separation. There may be irregularities in this separation also. Fig. 19 shows two interphase nuclei organised in such a manner. There are a few univalents left out also. Occasionally, this equational division is regular (Fig. 11) and in Fig. 13 we see M II each plate having the diploid number. Though we have not seen stages later than this, it is presumed four diploid tetrads will be organised ultimately. Whether these pollen grains are viable cannot be said, but their origin would appear to be through double division. In normal sexually reproducing plants, this is rather rare. In apomictic forms, like *Hieracium* (Rosenbergh, 1927) equational splitting of all the chromosomes takes place in both the divisions, giving rise to diploid gametes. A few other cases are also known. Clausen (1926) found in *Viola* some of the univalents dividing twice. In Ribes (Meurman, 1928) found that in a few cells there was complete absence of synapsis and these univalents divided twice regularly to give gametes with diploid number of chromosomes. There were, however, no data to show that these were functional in producing polyploids.

There is extensive degeneration. Many assume a shrivelled up crescent-shaped form with univalents spread all through (Fig. 15).

(*c*) *Fusion of M II Plates.*—Fig. 14 shows a cell with 48 chromosomes. This could have arisen in either of two ways. If in a P.M.C. both the first and second divisions are interrupted, a nucleus containing a tetraploid number may be formed. This would be a restitution nucleus and it is not likely to have operated in the formation of the present cell. Firstly, such a nucleus would be of irregular shape and the size of the cell itself will be bigger. Moreover, in a restitution nucleus loss of a few chromosomes is

almost the rule and the fact that the entire 48 chromosomes are seen rules out the possibility of this cell having been formed in such a manner. The probability is that two M II plates each with the diploid number have fused and resulted in this tetraploid cell. Such fusions are reported to be very common in X-rayed *Nicotiana* (Goodspeed, 1929). In Fig. 53 the M II plates cannot be distinguished because of lack of proper orientation and the consequent even distribution of all the chromosomes.
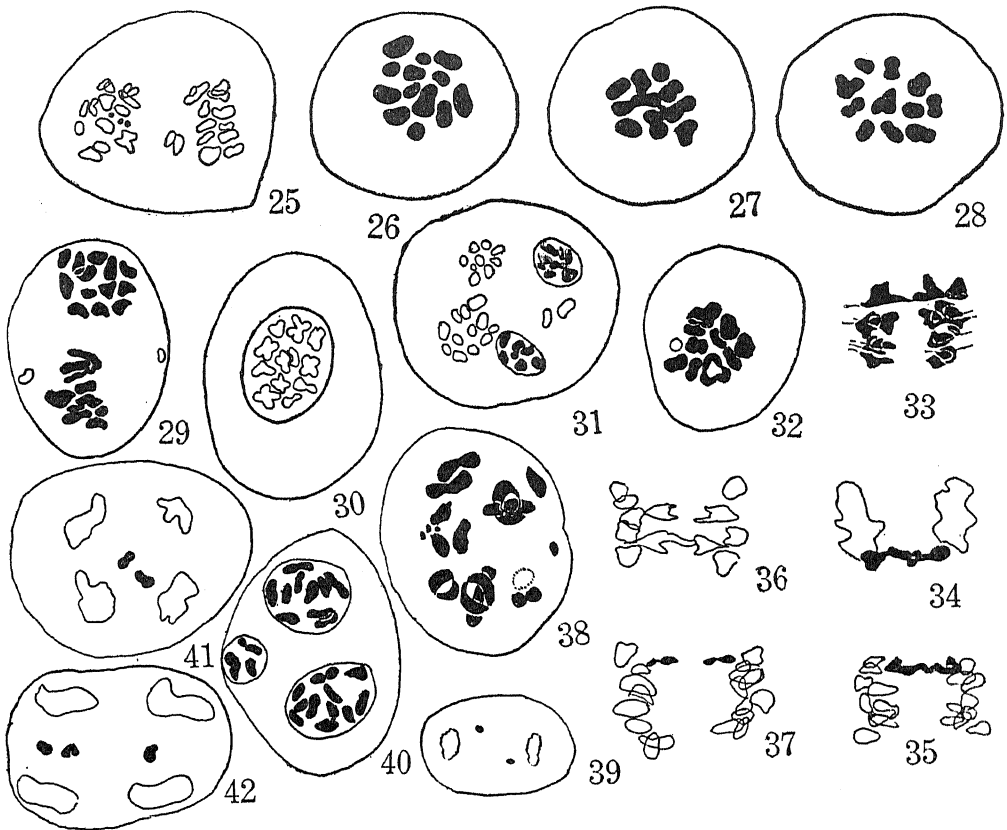
(d) Non-disjunction.—The phenomenon of bivalents reaching the poles without disjunction was commonly met with (Figs. 21 and 24 and Pl. I, Figs. 7–9). In such cases, these bivalents reach the poles earlier, where they naturally increase the number of chromosomes in the poles they reach. In Pl. I, Fig. 7, a ring bivalent has already reached the pole. In Pl. I, Fig. 9, a bivalent is recognised in telophase. It is only by such a method that we can explain the occurrence of 13 univalents in some cases. Fig. 24 is that of M I where the bivalent is reaching the pole earlier. In some cases, however, it would appear that these bivalents are not included in the poles so that

they remain as bivalents till the end, having been cast into the surrounding cytoplasm and not taking part in the divisions. They become lost. Presumably because of such losses, we get very often M II plates showing numbers smaller than 12. Fig. 23 shows 8/8 and in Fig. 22 9/9. It is interesting that we get the same number in each plate of a particular cell. This confirms the assumption that three bivalents in the one case and four in the other have not taken part in the division. Hence the deficiency.

Rarely these extruded bivalents have separate spindles which are very much smaller than the normal ones. In Fig. 51 we see a cell in anaphase I showing two spindles, the smaller representing the spindle of the bivalents which were off the plate. We also find very often chromosomes spread out and orientated in all directions. In Fig. 49 we find tripolar spindles in the first division. The frequency with which these occur shows that the spindle is a compound structure as suggested by Gates (1932).
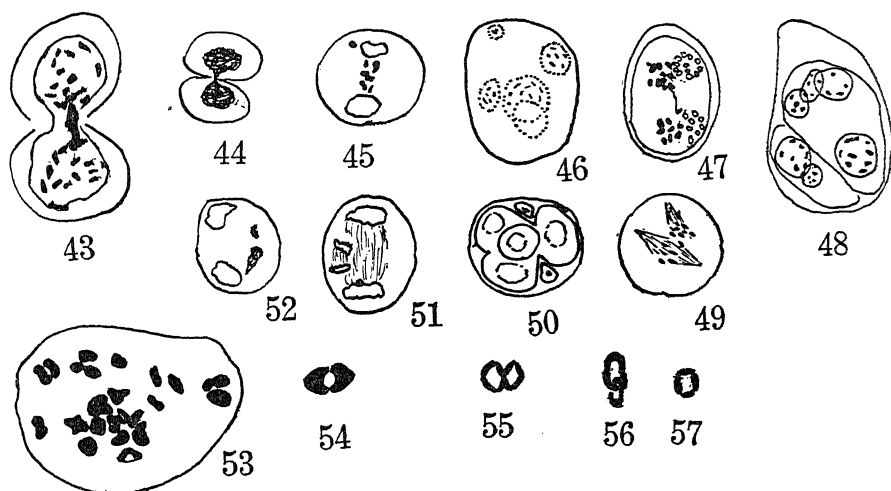
(e) *Interlocking.*—Occasionally at diakinesis the phenomenon of interlocking was observed (Fig. 56 and Pl. I, Fig. 14). The interlocking was either between two bivalents or between a ring of four and a bivalent. The interlocking is due to the peculiar distribution of threads at zygotene. When synapsis between two homologues is taking place, a third chromosome from another pair may come between the pairing threads and the formation of chiasmata on either side in the chromosome pairs will naturally result in the interlocking of bivalents in the succeeding diakinesis and metaphase. Interlocking has been reported to occur among others, in *Allium* (Levan, 1933).

(f) *Cytomixis.*—Frequently, cases of cytomixis have been met with and this at various stages of meiosis. Fig. 44 shows it at early prophase. Fig. 43

in diakinesis. This phenomenon was first described by Gates (1911) in *Oenothera*, where he observed the transference of chromatin between two adjacent cells, through gaps in the cell walls by means of protoplasmic connections. This phenomenon has since been reported in many plants. Kattermann (1933) gives a list of these works and describes cytomixis in P.M.C. of *Triticum* × *Secala* hybrids. Though in the present case, cytomixis is confined to the first division stages, cases are known where, this takes place in the second division also. Gates and Latter (1927) described such a behaviour in various stages of meiosis. It is not unlikely that in the present case where, cytomixis is frequent, the result is an increase in chromosome number. The cell with 48 chromosomes was interpreted to have arisen by the fusion of M II plates. Such cells may also arise by the passage of the whole of the nuclear contents from one mother-cell to another. Such a passage has been reported before and in rice Nandi (1936) found one case in which both the nuclear contents passed from one mother-cell to another and forming a binucleate P.M.C. at diakinesis.

(g) *Ring Formation.*—Though bivalent formation is more or less the rule, some cases were met with where rings were seen to be formed. Fig. 57 and Pl. I, Fig. 17 show a ring of four at diakinesis. Afify (1933) found in *Aconitum* rings forming very occasionally, the normal behaviour being regular bivalent formation. This shows that only small segments have interchanged. The association of four chromosomes in the semi-sterile plant which being a diploid, forms normally only bivalents, is due to interchange of segments between non-homologous chromosomes as first suggested by Belling (1925). In trisomic *Daturas*, Belling and Blakeslee (1926) found ring formation and inferred the occurrence of an interchange of segments between non-homologous chromosomes. This has since been used in interpreting ring formation in plants arising from hybridisation or irradiation and also in naturally occurring forms. Chromosome ring formation was first recorded by Gates (1908) in *Oenothera rubrinervis*. Parthasarathy (1938) found ring formation in the X–1 generation of X-rayed rice seeds and this was exhibited also by a semi-sterile plant as in the present case. Fig. 55 and Pl. I, Fig. 16 show orientation of this ring prior to anaphasic separation and it appears that disjunction will be of the AB, CD, and BC, DA type giving viable gametes. Random orientation also occurs disjoining on the AB, BC, and CD, DA basis. And this would give rise to inviable gametes due to deficiency of D and B segments.

(h) *Other Abnormalities.*—A variety of abnormal meiotic conditions other than those already described include chromosome fragmentation, and lagging chromosomes. Fig. 39 shows two chromosomes lagging in division I,

Figs. 41 and 42 show them at division II. Occasionally we find chromosomes lagging throughout the spindle (Fig. 45). Often in diakinesis, we get bodies less than 12 in number, due to formation of trivalents (Figs. 28 and 32 and Pl. I, Fig. 11). Consequently, a variable number of univalents also arise. In Fig. 26 and Pl. I, Fig. 13, we see 13 bodies, 11 bivalents and 2 univalents. There is also a marked tendency for these bodies to fuse (Fig. 27 and Pl. I, Fig. 15). The behaviour of the univalents is very irregular. Some are extruded; they either get lost or organise themselves into cells of their own (Fig. 40). On account of irregular divisions groups of univalents of varying numbers are formed and each group comes to be invested by a wall, and the result is a number of cells of different sizes are formed. Fig. 50 shows 6 such cells, while Fig. 31 shows them at earlier stages leading to the ultimate formation of these different-sized cells. Anaphasic separation of the chromosomes is abnormal. A proportion separate normally, while the remainder lag. Fragments are seen sometimes (Fig. 38). Fig. 17 is A I with 13 chromosomes and a fragment. The separating chromosomes are drawn out into threads (Figs. 34–36) and this is common in second division also (Fig. 47). The bulk of the chromatin reaches the poles but some remain in the plate and disintegrate. In Fig. 52 masses of disintegrating chromatin are seen. At interphase, the chromosomes show a marked split. In Fig. 30 we see one of the interphase nuclei. Most of the bridges (Figs. 33–37) that are seen seem to be the result of the stretching of the chromosomes, on account of incomplete treminilisation of the chiasmata especially of the trivalents which are formed as an abnormality. Separation takes place in some cases so violently that the torn ends can be seen clearly (Fig. 36). It seems that none of these bridges is to be regarded as inversion bridges. The second division is equally irregular. In Pl. I, Fig. 10 *a*, we recognise two univalents lying off the M II plates, while in Fig. 41 two univalents are lagging in second telophase. There is also indication of non-simultaneous division. In Fig. 25 two univalents are lagging and in one pole can be recognised three fragments. In Fig. 29 and Pl. I, Fig. 10 *a* which is a M II, two univalents are off the spindle. In one pole there are 13 chromosomes and the other 10. Obviously, the two bodies lying outside the spindle cannot be two univalents but the product of the division of a single univalent, which must have been cast out in division I. Laggings in second division are represented in Figs. 31, 41 and 42.

VII. General Considerations

Experiments on the application of radiation to plants have been carried out by numerous workers. Goodspeed (1929), Goodspeed and Avery (1930)

have described chromosome alteration induced by X-rays in *Nicotiana*, Katayama (1935) in wheat, Catechside (1935) in *Oenothera*. The cytological changes involved were found to be gene mutation, translocation, inversions and deficiencies and these were later confirmed by genetical results. Most of these induced mutations were useless from the economic point of view. The changes induced are so varied that the possibilities of obtaining desirable types are great, though it must be admitted that the results cannot be anticipated. In the present case, most of these gene mutations except inversion have been described and an interesting mutant with erect fruits has occurred. Normally pendant is dominant to erect. Further progenies of these erects are being studied. In the meantime it is interesting to record that a similar mutation has been obtained by Colchicine pretreatment of seeds, perhaps a case of parallelism in mutation.

There would appear to be ample scope of obtaining polyploids through X-ray treatment in Capsicum. Besides asynapsis, which has been previously recorded, Cytomixis which has been described in the present paper would appear to play an important part in the establishment of polyploids. The importance of polyploids in plants of economic importance like chilly, cannot be over-estimated. In the words of Blakeslee (1937)· · · ·" It is our belief that in the future extra chromosomes will be consciously utilized as a source of desirable variations in plants of economic importance. · · · · · To the geneticist who is specializing in any economic form and who desires to be most practical, our advice would be ' know your chromosomes '."

VIII. Summary

A number of varieties of South Indian Chillies have been described. They are all found to be varieties of *Capsicum annuum*. All have $2n = 24$. The somatic complement of two widely differing types have been analysed and it is suggested structural rather than numerical and morphological variations have played a part in the evolution of the various varieties. Meiosis is found to be regular. Seeds from a pure line of Paramakkudy were exposed to X-rays and mutants in X-1 and X-2 generations have been isolated. Meiosis in a semi-sterile mutant of the X-2 generation is described. It is found to exhibit asynapsis, cytomixis and chromosome ring formation. A number of other meiotic abnormalities are also described. The importance of X-ray treatment in the evolution of polyploid forms either through asynapsis or through cytomixis is indicated.

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EXPLANATION OF PLATE I

FIGS. 1-6: PHOTOGRAPHS

FIG. 1.—Photograph of ' Bushy erect ' (Pa X–4)

FIG. 2.— ,, ' Dwarf ' (Pa X–6)

FIG. 3.— ,, ' Lean erect ' (Pa X–3).

FIG. 4.— ,, ' Narrow-leaved giant ' (Pa X–2).

FIG. 5.— ,, ' Nigroides ' (Pa X–5).

FIG. 6.— ,, ' Large-leaved giant ' (Pa X–1).

FIGS. 7–17: PHOTOMICROGRAPHS

FIGS. 7–9.—Non-disjunction; note the ring bivalent going to the pole without disjunction.

FIG. 10—M II with one univalent off the spindle.

FIG. 10a.—One of the plates in polar view and two univalents extruded.

FIG. 11.—M I showing eleven bodies.

FIG. 12.—First telophase showing formation of bridge through delayed disjunction of trivalents.

FIG. 13.—First metaphase, eleven bivalents and two univalents.

FIG. 14.—Interlocking; same as Text-fig. 56.

FIG. 15.—First metaphase-bivalents extruding tendency to fuse; same as Text-fig. 27.

FIG. 16.—Ring of four prior to disjunction; similar to Text-fig. 55 which is however another ring.

FIG. 17.—Ring of four and also a chain; same as Text-fig. 57.

TEXT-FIGS 1–57

Text-figs 1–7.— × Ca 3,300 except Figs. 5 and 6 which are × 2,500.

FIGS. 1 and 2.—Idiogram and somatic complement of ' Paramakkudy '.

FIGS. 3 and 4.—Idiogram and somatic complement of ' Sunnybrook '.

FIG. 5.—M I Paramakkudy, 12 bivalents.

FIG. 6.—Interphase; note prominent constrictions of the chromosomes.

FIG. 7.—M II; both plates polar view 12/12.

Text-figs. 8–24.— × *a* 1,650 except Fig. 16 which is × 2,500; Figs. 21–24 × 3,300.

FIG. 8.—Asynaptic P.M.C; note complete failure of pairing and the consequent diploid number of univalents.

FIG. 9.—Asynaptic P.M.C., with only one ring bivalent.

FIG. 10.—Organization of a monad with 2 *n* chromosomes.

FIG. 11.—Interphase regular equational divisional of the 2 *n* P.M.C.

FIG. 12.—Random assortment of univalents.

FIG. 13.—M II with 2 *n* number in each plate—a result of regular equational divisional division.

FIG. 14.—Mother cell with about 48 chromosomes, presumed to have arisen by the fusion of M II plates.

FIG. 15.—Shrivelled up crescent-shaped cells with univalents spread all through.

FIG. 16.—Mother cell showing 48 bodies derived by the splitting of each univalent.

FIG. 17.—First anaphase, 13 chromosomes and a fragment.

FIG. 18.—Early stage in the assortment of univalents into groups prior to organization of irregular nuclei.

FIG. 19.—Interphase nuclei organized by irregular division of a 2 *n* P.M.C. Note some univalents left out.

FIG. 20.—Another type of irregular division of an asynaptic P.M.C.

FIG. 21.—First telophase with two bivalents extruded.

FIG. 22.—M II polar view nine in each plate.

FIG. 23.—M II polar view eight in each plate. Both these have arisen by the deletion of bivalents.

FIG. 24.—M I side-view; note one bivalent migrating to a pole earlier than the rest without disjunction; same as Plate I, Fig. 7.

Text-figs. 25–42.— × Ca 3,300 except Fig. 40 which is × 1,650 and Fig. 42 which is × 2,500.

FIG. 25.—M II. Note two univalents un-included and the presence of three fragments.

FIG. 26.—M I with 13 bodies.

FIG. 27.—M I; same as Plate I, Fig. 15.

FIG. 28.—M I eleven bodies; same as Plate I, Fig. 11.

FIG. 29.—M II two univalents off the spindle, presumably the product of the division of a single univalent.

FIG. 30.—Interphase, 12 chromosomes with deep constrictions.

FIG. 31.—Univalents, assorting irregularly to form different sized cells.

FIG. 32.—M I eleven bodies; note the trivalent and the univalent.

FIGS. 33–37.—Various types of bridge formation owing to delayed disjunction of trivalents.

FIG. 38.—Diakinesis with 13 bodies and three fragments.

FIG. 39.—First telophase with two lagging univalents.

FIG. 40.—Irregular behaviour of the univalents; three different sized cells are formed. This is a later stage than Fig. 31.

FIGS. 41–42.—Lagging chromosomes in the second division.

Text-figs. 43–57.—Figs. 43–52 × Ca 1,650; Figs. 53–57 × Ca 3,300.

FIG. 43.—Cytomixis at diakinesis.

FIG. 44.—Cytomixis at early prophase.

FIG. 45.—First telophase; no. of chromosomes spread throughout the spindle.

FIG. 46.—5 nuclei of different sizes are formed by the random assortment of the univalents of an asynaptic P.M.C.

FIG. 47.—Second division, separating chromosomes drawn into threads.

FIG. 48.—Same as 46, six irregular nuclei are formed but the division of the cell into two has taken place.

B1*a*

FIG. 49.—Tripolar spindle.

FIG. 50.—Six cells of different sizes formed by the random assortment and organization of the univalents.

FIG. 51.—P.M.C. in the first division, showing two spindles, the smaller spindle being that of the extruded bivalents.

FIG. 52.—First division shown extruded masses of degenerating chromatin.

FIG. 53.—Fusion of M II plates.

FIG. 54.—A typical ring bivalent.

FIG. 55.—Ring of four assuming the zig-zag configuration prior to disjunction.

FIG. 56.—Interlocking; same as Plate I, Fig. 14.

FIG. 57.—Ring of four; same as Plate I, Fig. 17.

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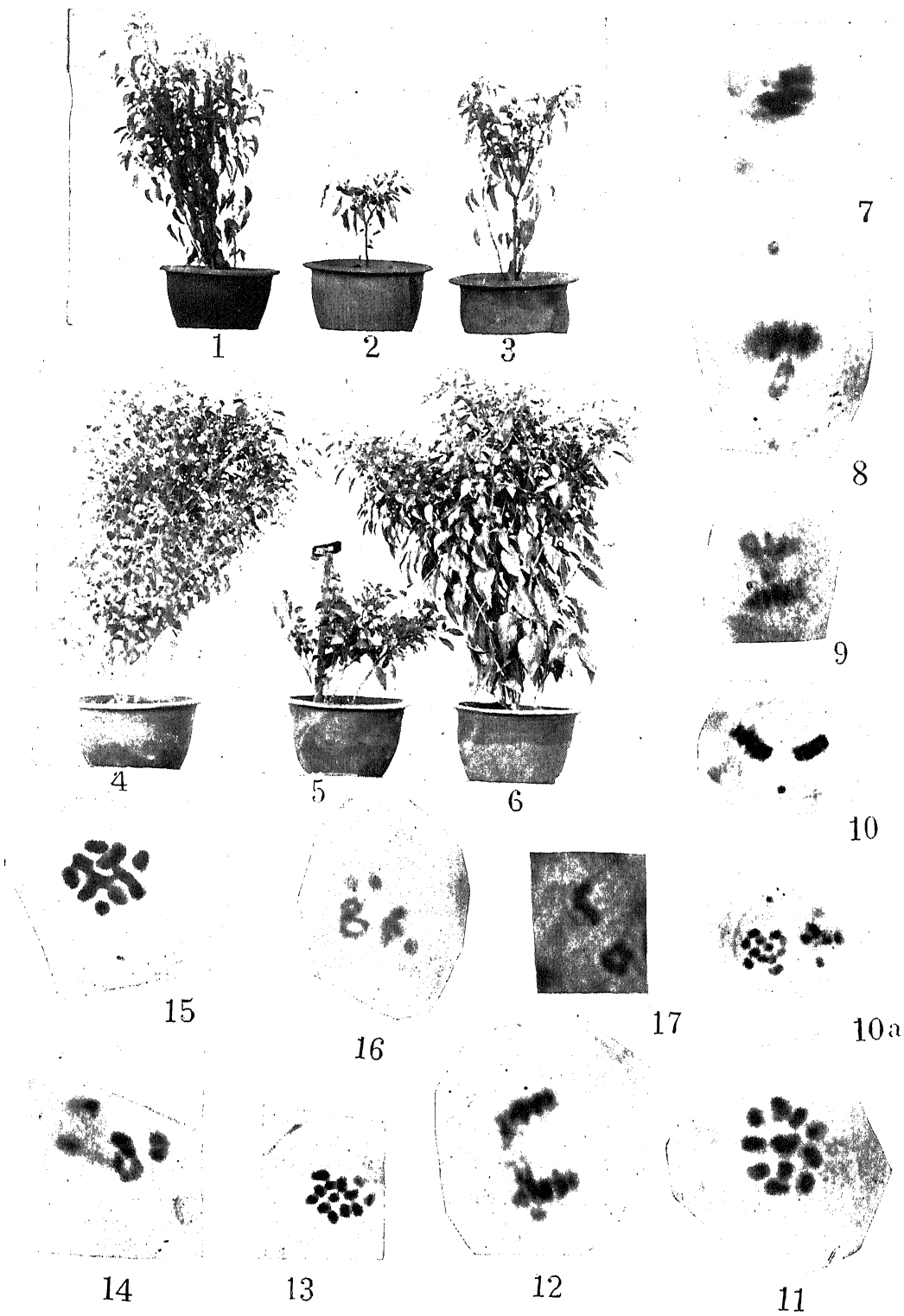
CHROMOSOMES OF *TYPHOPHTERA DONOVANI* DON. (TETTIGONIDÆ)

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1. Material and Method

Typhophtera donovani Don. is a very interesting Orthopteran insect furnishing a striking example of protective colouration. The colouration of its wings is very similar to that of a dead and decaying wet leaf, showing yellowish green patches of irregular outline on a brown or dark brown background with prominent venation. Specimens were obtained from small shrubs and trees near the hedges of cultivated fields in the vicinity of the Gujarat College, Ahmedabad, Western India. These were obtained in the latter half of August and early September during the monsoon of 1937, 1938 and 1939. They are by no means common and are not easily found. The author is greatly indebted to Dr. H. S. Pruthi, Imperial Entomologist, Agricultural Research Station, New Delhi, who identified these specimens and who has been doing similar favours from time to time which is of great assistance in our research work.

Specimens were killed by pouring a little xylol over them soon after they were obtained, testes were quickly dissected out in normal saline and fixed in two or three modifications of strong Flemming with considerably reduced proportions of glacial acetic acid, and in several cases with only a trace of it. Modifications of Flemming are obtained by diluting the stock solution at the time of fixing by adding distilled water in varying proportions. A fixative, for instance, containing only one part of strong Flemming to four parts of distilled water with only a drop of glacial acetic to every 5 c.c. of this fluid is equally effective. The material was left in the fixatives from about 20 to 24 hours, washed for an equal length of time and was dehydrated and cleared either in varying mixtures of dioxane and distilled water or ethyl alcohol and distilled water in graded series of $2\frac{1}{2}$ and 5 per cent. After it was embedded in paraffin, sections 12μ in thickness were cut, bleached, mordanted in $2\frac{1}{2}$ to 3 per cent. solution of iron alum for 25 minutes only, washed in running water 3 to 4 minutes and stained in $\frac{1}{2}$ per cent. hæmatoxylin for 2 to 4 hours or overnight.

B2   F

2. *Observations*

Chromosomes of Tettigonidæ are comparatively very small when viewed against those of Acrididæ. *Typhophtera donovani* Don. is the ninth form in the series of Indian Tettigonidæ so far examined (Asana, Makino and Niiyama, 1938). Figs. 1–2 are examples of many spermatogonial metaphase plates observed. 35 chromosomes have been counted in the equatorial plates. The chromosomal complex of this species as seen in the diploid stage resembles the chromosome complements of *Concephalus* sp. (Asana, Makino and Niiyama, 1938), *Xiphidion gladiatum* (Ohmachi and Sokame, 1935), *Orchelimum vulgare* and *O. concinnum* (King, 1924). It differs from them in the number of its chromosomes, there being 35 spermatogonial chromosomes in it, while the other forms have 33 (see Figs. 1 and 2). The spermatogonial chromosomes of *Typhophtera donovani* Don. form a motley garniture and consist of 18 minute spheroidal elements, 16 long and medium sized rods and a solitary X-chromosome which is the largest and is V-shaped. When we compare this diploid garniture of *T. donovani* Don. with the chromosomal complex of the same stage in *Concephalus* sp. studied by Asana, Makino and Niiyama (1938), it becomes evident that the latter form possesses in its spermatogonial complement two V-shaped autosomal chromosomes, while no such elements are found in the species which is the subject-matter of this paper.
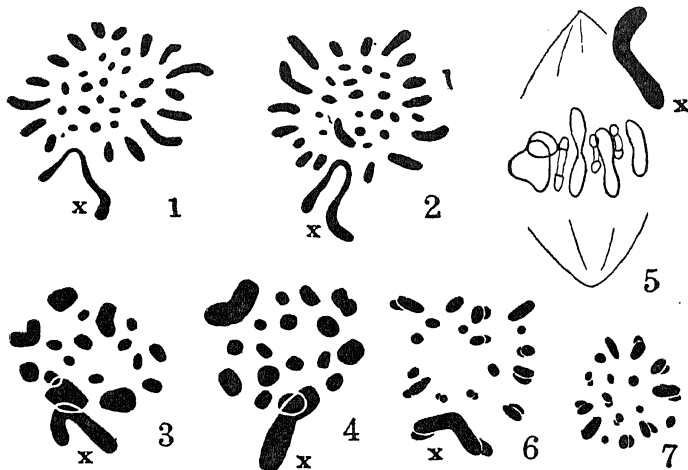
Chromosomes of *Typhophtera donovani* Don., Tettigonidæ (Orthoptera). × 3,500

Coming to the metaphase of the primary spermatocytes 17 bivalents and an X-chromosome are found in the equatorial plate as seen in Figs. 3 and 4. The X-chromosome at this stage has its apex always directed

towards one of the poles and runs ahead of the others (Fig. 5).   As a result of this division of the primary spermatocytes there are produced two kinds of secondary spermatocytes, one group have the x-element and the other are without it. This is clearly seen in the metaphase plates of the secondary spermatocytes as shown in Figs. 6 and 7 where a representative of one class (Fig. 6) has 18 chromosomes including the x-element, while a representative of the other class (Fig. 7) has no such chromosome in its complement.

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OVULE MORTALITY IN GRAM (*CICER ARIETINUM* L.)

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I. Introduction

IN the course of an investigation on seed-setting in gram (*Cicer arietinum* L.) the writers observed that some pods contained a number of shrivelled-up and undersized seeds, presumably as a result of the failure of some ovules to get fertilised or to develop normally after fertilisation. As this has an important bearing on yield, a study of the productive capacity of the plant in terms of the number of ovules produced and the number which develop into mature seeds was taken up. The work was conducted at Pusa in 1937–38.

II. Material and Methods

Twenty-eight varieties of gram were selected for the study, the selection being based on peculiarities of seed-setting observed in the previous season.

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These varieties could be classified into the following six classes on the basis of their seed-setting behaviour :—

1. Varieties predominantly one-seeded   .. 1*, 4, 38, 39, 42 and 29.
2. Varieties predominantly two-seeded   .. 9, 17, 25, 53, 57, 64.
3. Varieties with three-seeded pods   .. 47 and 58.
4. Varieties with three and four-seeded pods .. 76, 82, 83, 84.
5. Varieties with a relatively low number of
     empty pods   ..   ..   .. 12, 60, 61, 73, 74.
6. Varieties with a relatively high number of
     empty pods   ..   ..   .. 3, 13, 20, 22, 30.

A maximum of two hundred pods of a fair size were collected from ten randomly selected plants of each variety. They were of all ages but the distinction between the developing and aborted ovules was clear.

III. Variation in the Production of Ovules

The variation in the number of ovules per pod which was observed, is given in Table I. The maximum number of ovules found in a pod is four (with but one exception where five ovules were found) but this is not common. Two-ovule pods are the most numerous; three-ovule pods are also common but pods with a single ovule are rare. All variations in the number of ovules per pod may be found within the same variety, but the proportion in which the different ovule groups occur is evidently a varietal characteristic. Some varieties show a predominance of one particular ovule group while in others another ovule group may be more common, *e.g.*, varieties numbers 82, 83 and 84 have more than 20 per cent. of four-ovule pods, whereas in the other varieties the proportion of four-ovule pods is very low. Again, in eleven varieties, two-ovule pods form over 90 per cent. of the total.

It is interesting to note that three- and four-ovule pods are more widely distributed among the varieties than would be suspected from a study of seed setting alone. In the seed-setting studies which were conducted by us (not reported here) varieties 82, 83 and 84 gave a few four-seeded pods but the present study of ovule production shows that the four-ovule group occurs in 18 out of the 28 varieties examined. Similarly three-ovule pods were found in all the 28 varieties.

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* The numbers refer to the varieties of gram bred at the Imperial Agricultural Research Institute. Thus variety 1 represents Imperial Pusa 1, and so on. They have been described by Howard, Howard and Khan (1915) and by Shaw and Khan (1931).

TABLE I

Variation in the Production of Ovules in Selected Varieties of Gram

Variety	Number of pods				Total pods	Percentage of pods			
	With 1 ovule	With 2 ovules	With 3 ovules	With 4 ovules		With 1 ovule	With 2 ovules	With 3 ovules	With 4 ovules
1	..	94	6	..	100	..	94·0	6	..
3	6	168	24	2	200	3	84·0	12	1
4	1	87	10	2	100	1	87·0	10	2
9	..	161	39	..	200	..	80·5	19·5	..
12	22	151	27	..	200	11	75·5	13·5	..
13	..	91	8	1	100	..	91·0	8·0	1
17	1	190	8	1	200	0·5	95·0	4·0	0·5
20	..	182	18	..	200	..	91·0	9·0	..
22	..	173	17	..	190	..	91·0	9·0	..
25	6	85	94	15	200	3	42·5	47·0	7·5
29	..	67	106	27	200	..	33·5	53·0	13·5
30	..	108	85	7	200	..	54·0	42·5	3·5
38	1	59	19	1	80	1·3	73·7	23·7	1·3
39	..	182	17	1	200	..	91·0	8·5	0·5
42	..	99	1	..	100	..	99·0	1·0	..
47	2	101	82	15	200	1·0	50·5	41·0	7·5
53	1	71	25	3	100	1·0	71·0	25·0	3·0
57	1	195	4	..	200	0·5	97·5	2·0	..
58	2	135	56	7	200	2·0	62·5	28·0	3·5
60	12	178	10	..	200	6·0	89·0	5	..
61	..	166	32	2	200	..	83·0	16·0	1
64	1	45	3	1	50	2·0	90·0	6·0	2·0
73	..	74	16	..	90	..	82·0	18·0	..
74	1	180	19	..	200	0·5	90·0	9·5	..
76	..	190	9	1	200	..	95·0	4·5	0·5
82	..	22	116	62	200	..	11·0	58·0	31·0
83	..	52	101	46+1*	200	..	26·0	50·5	23·5+0·5*
84	..	20	68	42	130	..	16·1	52·3	32·3
Total	57	3326	1,020	236+1	4640	1·23	71·68	21·99	5·1

* One pod had 5 ovules.

An analysis of ovule production in these varieties suggests that the latter differ in their inherent capacity to produce a high number of ovules. Only some varieties are able to produce four ovules per pod; even in these varieties the high level of ovule formation is limited to a certain proportion of the total number of pods produced.

IV. Abortion of Ovules

Many more ovules are produced than develop into seeds. The total figures for all the types are given in Table II.

TABLE II

Abortion of Ovules in Varieties of Gram

No. of varieties	No. of ovules aborted	No. of ovules set	Total No. of ovules initiated	Percentage setting	Percentage sterility
28	3,919	6,799	10,718	63·44	36·56

Taking all the varieties into consideration, only sixty-three out of a hundred ovules develop into seeds. But different varieties show different degrees of setting. The percentage setting* was found to vary from 50·4 per cent. in variety 39 to 85·9 in variety 20. This is a very wide variation and the ability to develop seed seems to be a varietal character. The four-ovule types are among the lowest setters (52–54 per cent.). Thus high ovule production does not mean high setting and hence types characterised by a high number of ovules per pod are not necessarily varieties of economic value. The so-called *Kabuli*† types are also low setters.

V. Setting in the Several Ovule Groups

The details of setting are presented in Table III.

The pods in each type were classified into two-, three- and four-ovule classes and the percentage of setting was calculated for each class. In some varieties the number of three- and four-ovule pods were too few for calculation of percentages. The percentage of setting is highest in the two-ovule group. It is less in the three-ovule group and least in the four-ovule group. Percentage setting in the one-ovule group was not calculated, the numbers being too few.

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* Kadam *et al* (1938) noted that the average setting in about twenty types of gram studied by them was about 53 per cent.

† We are informed by Dr. B. B. Mundkur, member of the Agricultural Delegation that visited Afghanistan in 1939, that gram is not grown anywhere around Kabul. The only area where it is grown on a small scale in Afghanistan is in the Laghman and Jalalabad valleys between the Sulaiman Mountains and the Hindukush Range.

TABLE III

Comparative Setting in the 2-, 3- and 4-Ovule Groups and in all the Pod Groups Combined

Variety	Total No. of ovules	% setting in all groups	% setting in the 2-ovule pods	% setting in the 3-ovule pods	% setting in the 4-ovule pods
1	206	58·73	59·57	50·0*	..
3	422	52·61	55·37	37·50	37·50*
4	213	62·44	64·37	56·70	37·50*
9	439	65·37	67·70	59·70	..
12	405	67·66	69·20	53·09	..
13	210	80·95	82·96	66·70*	75·00*
17	409	70·66	70·26	75·00	75·00*
20	418	85·88	87·10	77·70	..
22	397	72·54	73·69	65·92	..
25	518	60·61	70·60	58·16	40·00
29	560	55·18	66·42	53·46	46·30
30	499	64·54	74·99	56·85	53·67*
38	180	57·66	64·40	52·62	50·00 *
39	419	50·36	51·93	39·21	50·00*
42	201	55·22	55·55	33·36*	..
47	510	63·52	69·79	60·98	52·84
53	230	68·26	75·36	57·33	40·67*
57	403	72·95	73·58	50·00*	..
58	468	59·19	63·33	54·17	46·41*
60	398	62·05	62·90	46·67	..
61	436	67·20	70·49	56·23	62·50*
64	104	67·30	68·90	55·56*	50·00*
73	196	64·79	70·94	46·67	..
74	418	69·85	70·55	64·91	..
76	411	76·50	79·21	66·67*	50·00
82	640	52·81	72·73	55·75	45·15
83	596	62·85	66·34	52·16	46·56
84	412	54·24	75·00	57·85	45·83

* Based on less than 30 ovules.

In the four-ovule group it is usually below 50 per cent.   There is thus a far greater waste of plant material in the three- and four-ovule classes.   The two-ovule pods even among the four-ovule varieties like 82, 83 and 84 show a high percentage of setting (66-79 per cent.).   Within the two-ovule group there is great variation, being 51·9 per cent. in variety 39 and 87·1 in variety 20.   Considering the setting in all the groups, wide variations have been found, ranging from 50·4 in variety 39 to 85·9 in variety 20.

VI.   *Abortion in Relation to the Position of the Ovule in the Pod*

In the pod the ovules are arranged in a linear fashion one below the other.   The ovule nearest the stigmatic end has been referred to in this study as the topmost or first ovule, the one below being the second ovule, and so on.   The abortion of ovules in relation to the position occupied by them in the pod was studied and the results are given below.

The two-ovule group.—It is not always that both the ovules in a pod develop into seeds. Out of 3,326 pods examined only in 1,270 or 38 per cent. of the cases did both the ovules develop.   In nine cases both the ovules failed to develop.   In general, only one of the ovules develops, the other being aborted.   In all the varieties combined there were, 2,047 pods of the two-ovule group in which only one of the two ovules had developed. Of these, in 942 pods the first ovule and in 1,105 pods the second ovule was aborted. The first position therefore appears to be slightly more favourable.   Study-ing the varieties individually it was found that in eight the abortion at the first ovule position was greater and in three varieties the abortion was equal in the two positions.   The distribution of the fertile ovule in two-ovule pods is perhaps really at random.

The three-ovule group.—There were 1,020 pods in this group.

In the case of these three-ovule pods, the third or the bottommost posi-tion is definitely at a disadavantage.   The abortion in each position, in all the types combined, is as follows:—

Abortion in first ovular position	..	324
Abortion in second ovular position	..	348
Abortion in third ovular position	..	673
Total number of aborted ovules	..	1,345

Abortion thus appears to be nearly equally distributed in the first two positions, but the third position definitely predisposes to abortion.   It may be noted in passing that in 58 pods or nearly 6 per cent. of the pods, it was only the third ovule that developed, to the exclusion of the first two.   In 94 pods all the three ovules were found to have developed.

The four-ovule group.—The number of pods belonging to this group was only 236 and these were restricted in their distribution. Out of a possible 944 ovules only 435 or 46 per cent. developed and 509 failed to mature. The 509 undeveloped ovules in this group were distributed as follows :—

Abortion of first ovule	..	..	88
Abortion of second ovule	..	..	92
Abortion of third ovule	..	..	148
Abortion of fourth ovule	..	..	181
Total number of aborted ovules		..	509

In the matter of abortion the first and second ovules seem to fall into one group and the third and fourth ovules into another. The fall in fertility from the first to the second ovular position is almost imperceptible but from the second to the third is sudden and from the third to the fourth is only somewhat less so.

VII. Discussion

Ovule Production and Setting.—In this investigation an attempt has been made to study the factors influencing seed production and to examine the variations in the production of ovules which ultimately make up yield. Ovule mortality has been shown to be a very important factor which is significant in yield studies. Though four seeds per pod may theoretically be expected this is seldom realised in practice, and only about 60 per cent. of the ovules develop into seeds. Varietal differences have been found in ovule production. Increased production of ovules is however no indication of better setting and in fact the processes of production of ovules and their development into seeds appear to show an inverse relation, as shown in Table IV.

Harris (1913) also found an inverse ratio between ovule production and seed production in *Phaseolus vulgaris.*

A small number of ovules in the pod seems to favour better development. Taking the two-ovule group, we find in each variety wide variation ranging from 51·9 in variety 39 to 87·1 in variety 20 which suggests the existence of genetic factors. The present study reveals that there are two separate phenomena—the production of ovules, the capacity to develop them into seeds— which are probably genetic in basis.

The position of the fertile ovule.—Woodworth (1930) working on abortive seeds in soybeans found that the tip seed rather than the basal one has the better chance of development and concludes that seed development " may

TABLE IV

Percentage of Setting in Different Varieties of Gram

Group	Variety	Average No. of ovules per pod	Percentage of setting
4-ovule pods	29	2·8	55·18
	82	3·2	52·81
	83	3·0	52·85
	84	3·2	54·24
Many 3-ovule pods	25	2·6	60·61
	47	2·6	63·52
2-ovule pods only about 75 per cent. ..	12	2·0	67·66
	38	2·3	57·66
	53	2·3	68·26
	58	2·3	59·19
2-ovule pods above 90 per cent.	17	2·0	70·66
	20	2·1	85·88
	22	2·1	72·54
	42	2·0	55·22
	57	2·0	72·95
	76	2·1	76·50

be the expression of a general principle of reproductive development prevailing in plants". He does not consider nutrition to be the primary factor concerned in these differences. In the case of gram also the available evidence indicates the greater fertility of the tip ovules. In Table V the figures for

TABLE V

Position of the Aborting Ovule in Different Ovule Groups

Ovule group	Number of pods in which				Total No. of pods in the group
	The 1st ovule is aborted	The 2nd ovule is aborted	The 3rd ovule is aborted	The 4th ovule is aborted	
2	951	1,114	..	..	3,326
	*29**	*34*	..	..	
3	324	348	673	..	1,020
	32	*34*	*66*	..	
4	88	92	148	181	236
	37	*39*	*63*	*77*	

* The figures in italics give the percentage of pods with aborted ovules.

the number of pods in which the ovule is aborted in each position are summarised.

The ovules at the first two positions seem to stand an equal chance of fertilization. The diminution in fertility from the second to the third ovule position is sudden. The third ovule is infertile in 66 per cent. of the pods in the three-ovule group and 63 per cent. of the pods in the four-ovule pods. The fourth ovule is infertile in 77 per cent. of the pods. There is thus apparent a gradual decline in fertility from the first to the fourth ovular position. But the first two positions are more nearly alike and stand in the same class. The two lower ovule positions form another group.

Sterility, though greater in the lower positions, is by no means confined to them. There is considerable sterility in the first two positions. In the three-ovule group, in 5·7 per cent. of the cases (58 out of 1,020) only the third ovule develops, to the exclusion of the first two, and in the four-ovule pods, 11 per cent. of the pods (26 out of 236) show sterility of the first two positions.

Factors governing different growth rates of pollen tubes may be present, but this does not appear likely as in many cases ovules in the lower position develop to the exclusion of those in the first position. It is possible that "feeble incompatibility" factors may operate bringing about the abortion of ovules before or after fertilization.

Suppression of ovules.—The varietal differences in ovule production strongly suggest the operation of a genetic tendency for the gradual reduction in the number of ovules per pod. Though four ovules are not rare and three ovules are common, two ovules are the rule and the working out of this principle of reduction is further illustrated by the occasional production of one-ovuled pods.

Thompson (1929) has shown from a study of vasculation that this (reduction) is a phylogenetic tendency exhibited by the *Leguminosæ* as a whole. He has, in particular, drawn attention to the low ovule production in the family. Thus, out of 400 genera examined, there is but a single ovule in 28 genera while in 101 other genera, which includes *Cicer*, there are 1, 2 or few ovules. He concludes that "the gynaecium is unstable in both legume number and fertility".

Ovule abortion.—There are two aspects to this tendency for sterilisation; one is the complete elimination of ovules, the other is their abortion. The one represents the complete working out of the other tendency.

It is difficult to suggest an explanation for the failure of these ovules to develop. Defective pollen tube growth does not appear to be a reason. Though fertility is far less in the lower positions, the fact that in some cases only the ovules at the lower positions develop eliminates the possibility of defective pollen tube growth being the factor concerned.

Bradbury (1929), Dorsey (1919), Tukey (1933 and 1936) and Harrold (1935) studied ovular abortion in the stone fruits. They conclude that there is an abortion of the embryo or the embryo-sac and this is accompanied by a lack of proper nutrition. This explains *how* the failure of development comes about but not *why*. Martin (1915) attributes the abortion of ovules in alfalfa " to the fact that a few ovules monopolise the food supply". If it is merely a question of competition, either the lower ovules, which are nearer the sources of nutrition must develop, or the first ovule which is nearest the stigma and which may be expected to be fertilised first and thus get a start over the others must develop. The facts of ovule abortion do not support this. There are many cases of first ovule abortion and abortion in the lower positions is the greatest. Lack of nutrition as a probable explanation of ovule mortality has not been favoured by many investigators. Cooper *et al* (1937) studying ovule mortality in alfalfa agree with Kraus (1915) and consider that " the failure of conduction is the result rather than the cause of the failure of the growth of these parts". This is also in keeping with the known processes of vascular development of the leguminous carpel, where the greater part of the conducting tissue of the legume is laid down after, and in response to, fertilisation (Thompson, 1929). Maheswari (1931) thinks that the degeneration which commonly occurs in the embryo-sacs of *Albizzia lebbek* bears no causal relation to the nutritional disturbances but represents an inherent tendency. Mangelsdorf (1926) has shown that to obtain normal seed in maize 18 dominant factors must be present. Woodworth (1930) also concludes that the occurrence of abortive seeds in soybeans is largely a genetic problem.

In the case of gram, the failure to develop all the ovules appears to be the expression of a type of self-incompatability known as " reduced seed production". Our data do not permit any theorising but the known facts of ovule abortion support the view that it is fundamentally genetic in nature, though environmental factors also come into play.

VIII. Summary

1. The number of ovules per pod varies from one to four, and is a varietal character.

2. There appears to be a tendency in this crop plant for a gradual reduction in the number of ovules per pod.

3. All the ovules do not develop into seeds and the capacity to develop the ovules into seeds also varies considerably. It is higher in some varieties than in others. It is also higher in the two-ovule pods than in the three- and four-ovule pods.

4. Varieties which produce a larger number of ovules per pod are not necessarily those which set a high proportion of seeds.

5. The two ovules nearest to the stigma stand a better chance of development than those at the third and fourth positions.

6. It is suggested that the production of ovules as well as the capacity to develop them into seeds is due primarily to inherent causes, and, as such, is capable of genetic elucidation.

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A PRELIMINARY NOTE ON THE X-RAY MUTANTS OF PUSA (52) WHEAT

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Introduction

ACCORDING to the fourth and final report on the wheat crop for 1939–40 in U.P. nearly 81,37,452 acres were under wheat cultivation. This constitutes about 22 per cent. of the total area under wheat cultivation in India, and the out-turn of wheat is estimated to be 52,000 tons. The United Provinces is thus one of the important provinces with regard to the wheat crop of the country. The experiments hereinafter described were begun in an attempt to bring out some improved strains of wheat, suitable for the United Provinces.

Growing on various wheat tracts of the world there are possibly over 10,000 strains of wheat. For various reasons all these strains do not thrive equally well in all the countries and the usual method of hybridization is employed to produce specific strains of wheats suitable to a particular area. For instance, wheats of the Panjab, which are supposed to be superior in certain respects to the wheats of the U.P. do not thrive so well in the United Provinces.

Triticum vulgare, according to Percival,[5] is a vast collection of mutants and hybrids, which originated from a cross between *T. dicoccoides* and *Ægilops cylindrica*. On the other hand, McFadden (1930) thinks that the modern wheats are descended from certain wild wheat-like grasses.

Wheats thus being mutants and hybrids struck me to be a very suitable material for subjecting to X-ray treatment to produce fresh mutants. The word 'mutant' here is used in a wider sense and includes both 'gene mutations' and 'chromosomal aberrations'; though strictly speaking 'gene mutation' implies a hypothetical change within a gene. And, as 'gene' itself is invisible this type of germinal variation is only a matter of inference. A chromosomal variation can, however, be directly observed. In this preliminary work cytological studies have not been undertaken, so it will not be possible at this stage to say whether the mutants are a result of 'gene mutations' or 'chromosomal variations'.

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A search of available literature on the subject revealed very little of work on the effect of X-rays in producing new mutants.

Delaunay[1] X-rayed the ears of *T. vulgare albidum* and got 8 variant types. Six of these were found to be due to chromosomal aberrations. And 2 were supposed to be due to 'gene mutations'. Sapehin[7] as a result of X-rays found numerous variant types in his wheats.

Plotnikowa[6] X-rayed heads of wheat during meiosis and found that the treated material showed much disturbance in that the chromatin was clumped along the spindle threads. Certain of the cells also got multi-nucleated.

Delone[3] working on bearded and beardless varieties of spring wheats found that X-ray caused sterilization of the ears—the degree depending upon the stage of growth at which the ears were X-rayed. He also found that X-ray retarded growth.

Sapehin[8] (1935) working on *T. durum* found that X-rays produced changes in the external morphology, and a few F_1 plants studied cytologically showed abnormalities.

Experimental Procedure

Pure strain of Pusa (52) seeds, which were kindly sent to me by Dr. S. B. Singh, ph.d. (Cantab.), of the Agricultural Department, were germinated on moist filter-papers, and, when the radical had just come out, they were X-rayed.

In other cases Pusa (52) seeds were sown in pots and when the plants were about 14 days old, they were also exposed to X-rays. Immediately after such exposures, the seedlings were sown in previously marked out plots (10′ × 6′) in the Botanical Garden.

The most appropriate time for X-ray exposures was found to be at about 11 A.M., for this was the period at which the cells showed the greatest mitotic activity.

The seedlings to be X-rayed were taken to the X-ray room of the Physics Department of this University and exposed to X-rays from a distance of one foot from the anti-cathode of the Gas X-ray tube which was worked at 0·4 milliampere and 35 Kilowatt. The anti-cathode was of tungsten.

The period of exposure varied from 3–10 minutes in different sets of seedlings.

As a control, in an adjoining plot in the Botanical Garden, untreated seedlings of Pusa (52) were planted at the same period as the treated ones.

The plants were allowed to grow under usual agricultural conditions and soon after the ears started to mature a careful examination of the treated and untreated plants was made. The untreated, control plants were found to grow true to form, except in one or two cases in the whole plot where awnless-heads of the Pusa (4) type was found. These cases were probably due to a mixing up of a few seeds of Pusa (4) with the (52) variety before they were sent to us from the Government Agricultural Farm. On the other hand, the X-rayed plants, here and there, showed abnormalities in their ear-heads. The vast majority of the treated plants, however, showed no change in their external morphology.

The plants showing abnormalities were carefully marked, and after the ears had ripened, these plants were singly harvested.

Plate II shows eleven types of plants selected. There are 4 awned types and the rest awnless—the degree of awnlessness varying from short awns to no awns. A typical ear of Pusa (52) is also shown for the sake of comparison.

A few features of the external morphology of these mutant ears is given in Table I.

TABLE I

Observations on X-rayed Mutant Ears producing F_1 Wheats

Plant No.	Length of ear (cm.)	Density of ear $D = \dfrac{N \times 10}{L}$	Awned or not, etc.
1	6·9	26	Awned progressively. ↑
2	6·6	22	A few glumes have half-developed awns
3	5·0	22	Awnless
4	6·0	23	Awnless
5	6·8	19	Awnless
6	5·5	25	(Furred) Awnless. (A few glumes have rudimen-tary awns)
7	7·5	26	Awned progressively ↑
8	5·5	23	Awnless. (A few glumes have very rudimentary awns)
9	6·4	23	Awned
10	5·3	24	Awned progressively ↑
11	4·2	23	Awnless. (One or two glumes have very rudimen-tary awns)

The above 11 types of mutant ears containing the selfed F_1 seeds were carefully kept and were again sown the following year in separate small plots.

Plate III shows the 11 types of F_2 seeds, in small tubes, along with their ears.

A superficial comparison of the ears from which F_1 and F_2 seeds were taken shows that except for the size of the ears being larger in the F_2 and the density being greater in the F_1 (see Tables I & II) all the 11 strains behaved true to the new mutant types.

Table II gives some of the external morphology of the ears from which the F_2 seeds were taken.

TABLE II

Observations on Plants producing F_2 Wheats

Plant No.	No.* of tillers	Maximum ht.* of plants	Evenness of ht. (10)	Wt.* of grain per earhead	Wt.* of grain per plant	Length of ear* (cm.)	Density of ear* $D = \dfrac{N \times 10}{L}$	Awned or not, etc.
				gr.	gr.			
1	13	3′8″	4	1·1	21·22	7·8	20	Awned progressively ↑
2	10	2′3″	6	1·2	12·44	8·1	19	A few glumes have half-developed awns
3	12	3′4″	6	2·5	26·7	8·2	20	Awnless
4	20	3′5″	6	1·1	24·3	8·3	18	Awnless
5	17	3′4″	7–8	2·2	28·7	11·4	15	Awnless
6	17	3′7″	4	1·5	21·9	9·2	20	(Furred) Awnless. (A few glumes have rudimentary awns)
7	19	3′1″	7–8	2·0	37·5	9·3	18	Awned progressively ↑
8	14	3′1″	5	1·4	13·9	7·4	18	Awnless. (A few glumes have very rudimentary awns)
9	11	3′1″	4	2·0	18·1	8·0	21	Awned
10	9	3′2″	6	3·0	unknown	10·0	19	Awned progressively ↑
11	18	3′1″	6	1·6	22·4	10·2	19	Awnless. (One or two glumes have very rudimentary awns)
Pusa 52	10–19	2′8″–3′6″	8–9	2·2	18·2	9·3	23	Awned

* These data require to be treated statistically.

Summary

As a result of X-ray treatment of young seedlings of Pusa (52) wheats, eleven mutant types were separated. Of the eleven, 4 are awned types and the rest awnless—the degree of awnlessness varying from short awns to no awns.

The spikes of the mutants, in the first year, are much smaller than the spikes of Pusa (52). This is probably due to the disturbances caused by X-ray treatment of the seedlings. The spikes of all the 11 types of mutant, show a much greater length in the next generation.

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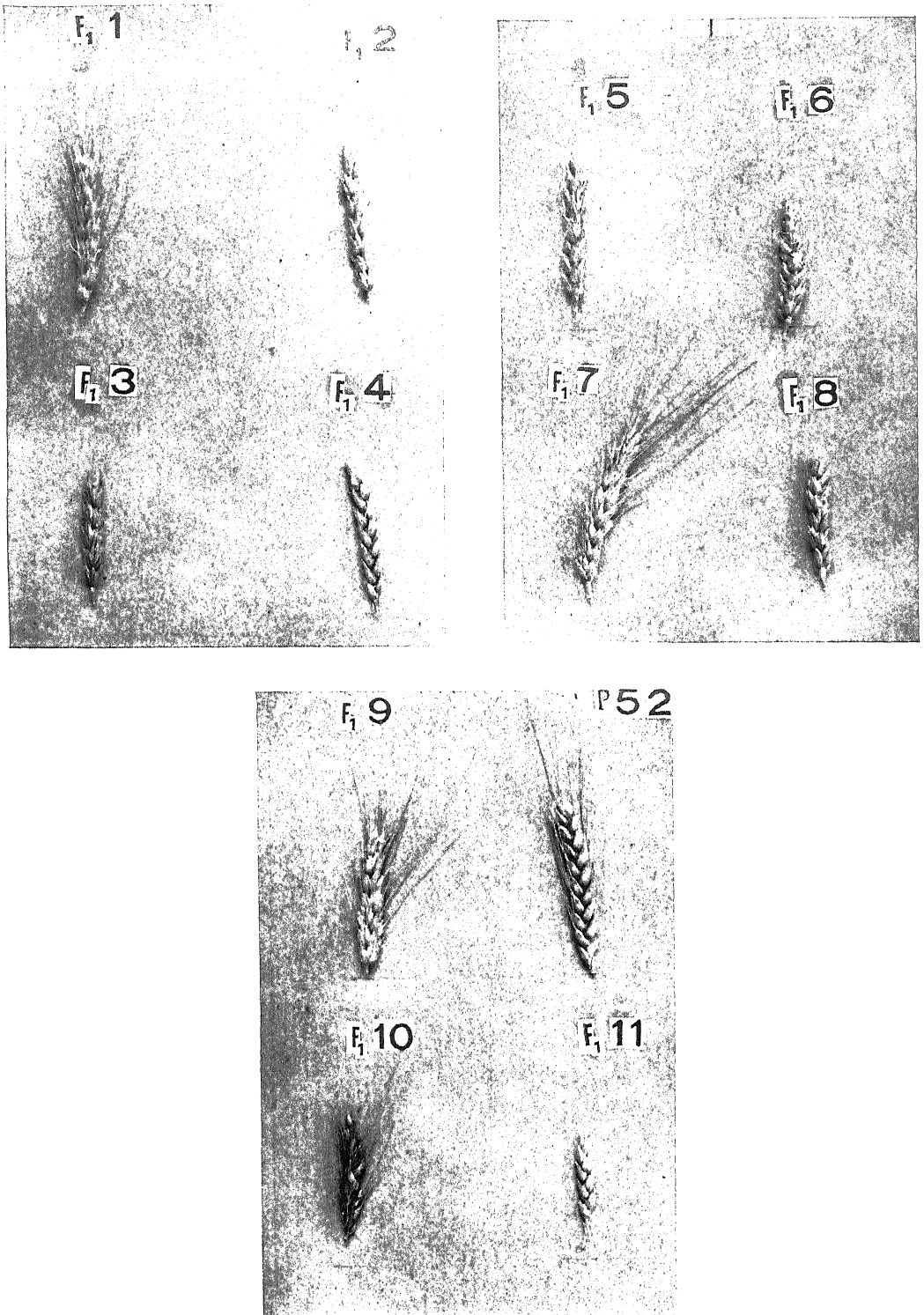
WHEAT. X-RAY MUTANTS OF PUSA (52)

The various mutant ears (F_1^1 to F_1^{11}) obtained after treatment of P (52)
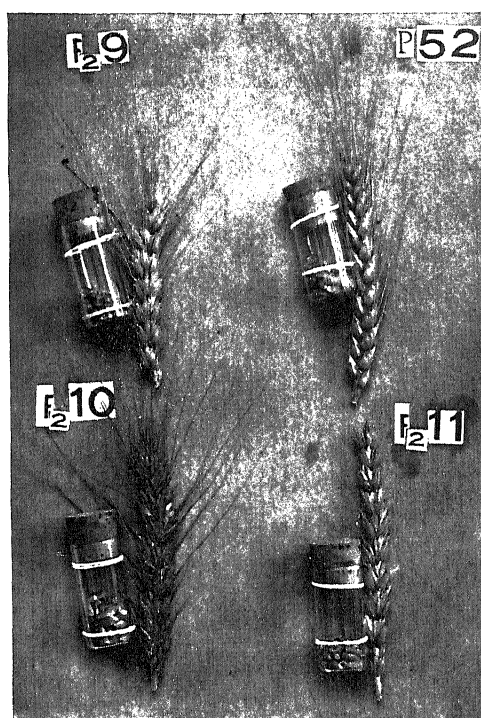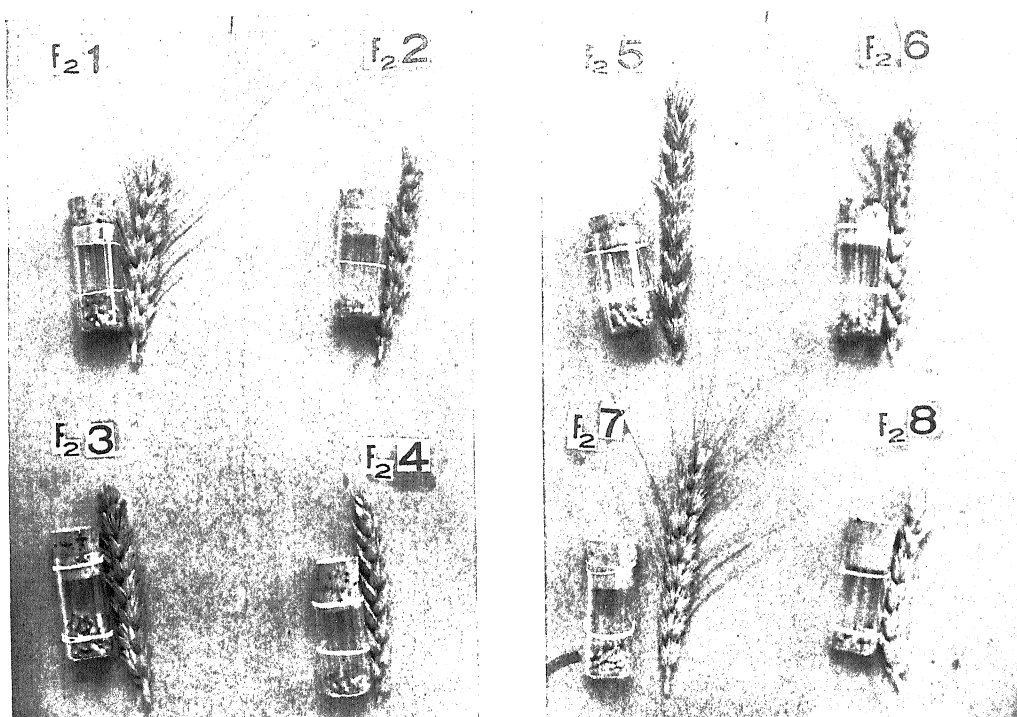
WHEAT. X-RAY MUTANTS OF PUSA (52)

Ears of the progeny obtained by selfing the mutants shown in Plate II, with their seeds in tubes ($F_2{}^1$ to $F_2{}^{11}$).

IMPORTANT INSECT PREDATORS OF INDIA

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CONTENTS

Introduction

INSECT pests of our country constitute a very serious problem. According to Sir Walter Morley Fletcher (Afzal Husain, 1938), " at a very modest estimate, the losses caused to India by insects that attack crops, timber and animal products cannot be less than 200 crores of rupees and a loss of over a million and a half of human lives ".

The injurious insects attack in amazingly large numbers: 85,400 adults of the notorious *Aspongopus janus* F. were collected from an area of 8 " marlas " (66′ × 33′) of " kaddus " (*Lagenaria vulgaris* Seringe) by 20 men in 10 minutes on the 2nd of September, 1939, at Lyallpur. To prevent the injurious insects from becoming unduly abundant and destructive Entomologists have developed four arms of defence, *viz.*, mechanical, cultural, chemical and biological. The work on the first three arms of defence has been in progress now for a number of years, but the work on the biological control of insect pests is of recent growth particularly in India.

The biological control is based on the assumption that a number of insect pests are destroyed by their insect enemies. Such insects are groupable into (1) parasites and (2) predators. Both these types of insects, rivalling each other in importance, rank among the Zamindars best and true friends. Of the two, predators are more numerous and more widely distributed than the parasites, and they show a greater range of adaptability (Thompson, 1929).

In India (as elsewhere) the insect predators have not been studied as thoroughly as their utility and economic importance would warrant. Further,

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the little information that exists about them is scattered in the literature on Indian Entomology. In this article it is proposed to bring all this information together and to add to it the knowledge gained about them in the Punjab[1] most of which has not yet seen the light of the day. It is hoped that this article will stimulate interest in the much neglected but very much useful insect predators of our country.

Insect Predators

I. COLEOPTERA[2]

I. *Coccinellidæ.*—

(1) *Coccinella undecimpunctata* L.—This beetle has been recorded from Behar and the Punjab; in the Punjab it is widely distributed.

During June its life-cycle is completed in 6–11 days as follows: egg stage, 1–2; larval stage, 4–6; pupal stage, 1–3. A female lays about 90 eggs in her life-time. These eggs are laid in clusters by instalments. The newly emerged adults can stand starvation for 24 hours.

C. undecimpunctata L. has been recorded feeding on wheat aphis (*Macrosiphum granarium* Kirby), cotton aphis (*Aphis gossypii* Glover) and mustard aphis *Rhopalosiphum pseudobrassicæ* Davis (*Siphocoryne indobrassicæ* Das). Each grub, depending upon its age, feeds on 30–80, and an adult on 30–105, aphides daily.

(2) *Coccinella septempunctata* L.—This is a widely distributed beetle in India which is active in winter only: It spends the period from April to October hiding among dense grasses and other sheltered places. During the active period its life-cycle is completed in about 50 days.

It feeds on wheat aphis, mustard aphis and cotton aphis as well as on the nymphs of the mango-hoppers (*Idiocerus atkinsoni* Leth. and *I. clypealis* Leth.).

(3) *Coccinella repanda* ab. *transversalis* F.—This beetle is fairly widely distributed in the Punjab and South India. It is active during winter when it feeds on Aleyrodidæ and Aphididæ.

(4) *Coccinella 7-punctata* ab. *divaricatae*.—This beetle has been recorded feeding on the nymphs of *Idiocerus* spp. from Hoshiarpur (Punjab) and Pusa (Behar).

(5) *Chilomenes sexmaculata* F.—This is the commonest of the Lady-bird beetles which is widely distributed in India. It is usually found in company with *Coccinella septempunctata* L. and *C. repanda* ab. *transversalis* F.

Each female can lay 100 eggs. In South India its life-history is completed in 15–19 days as follows; egg stage, 2–3; larval stage, 10–12; pupal stage 3–4. It feeds on Aleyrodidæ, Aphididæ and Coccidæ injurious to crops and fruit trees. Its larvæ and adults are said to be cannibalistic, for when food is scarce, they feed on their own eggs and larvæ.

(6) *Chilomenes bijugus infernalis* Muls.—This is a wedge-shaped, metallic-black small beetle which has been found feeding on Woolly aphis (*Eriosoma lanigerum* Haus.) and occasionally on San José scale. [*Quadraspidiotus* (*Aspidiotus*) *perniciosus* Comst.] in the Kulu valley (Punjab). It is usually very abundant during May–July when it destroys Woolly aphis in large numbers. It is parasitized by a Eulophid, *Tetrastichus* sp. which affects appreciable reduction in its numbers at a time of the year (August–November) when its presence is most needed to check multiplication of, and damage by, Woolly aphis.

(7) *Brumus suturalis* F.—This is a very widely distributed Lady-bird beetle in India which is active throughout the year.

It feeds on (1) eggs of sugarcane Top borer (*Scirpophaga nivella* F.: Pyralidæ) and sugarcane leaf-hopper (*Pyrilla perpusilla* Walk.: Fulgoridæ); (2) adults of the following Aleyrodidæ: *Trialeurodes ricini* Misra, *Dialeurodes citri* Ashm., *Bemisia gossypiperda* M. and L. and *Aleurocanthus woglumi* Ashby; (3) adults and nymphs of *Aphis gossypii* Glover (= *Aphis malvæ* Koch.) (Aphididæ); and (4) male puparia of *Phenacoccus insolitus* Green (Coccidæ).

(8) *Scymnus coccivora* Ramakrishna.—This is a tiny little beetle which feeds on the eggs of the " Nim " Mealy scale (*Pulvinaria maxima* Green). It is said to be very efficient in controlling the pest particularly during the hot summer months.

(9) *Scymnus gracilis* Mots.—This beetle has so far been recorded from Lyallpur and Coimbatore only.

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[1] K. B. M. Afzal Husain, Entomologist to Government, Punjab (at present Vice-Chancellor of the University of the Punjab, Lahore), initiated research on insect predators in the Punjab as far back as 1920. This work was continued under his supervision and guidance upto 1938. During this period much useful information was collected most of which lies buried in the Office files of the Entomologist to Government, Punjab, Lyallpur. I have made free use of this information in writing this article.

[2] I am grateful to the Forest Entomologist, Dehra Dun, for his kindly checking the spellings of the names of Coleoptera and to the Imperial Entomologist, New Delhi, for his kindly checking the rest of the names of insects mentioned in the article.

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(9) *Scymnus gracilis* Mots.—This beetle has so far been recorded from Lyallpur and Coimbatore only.

At Lyallpur it remains active during March–November and hibernates as an adult during December–February. During the active period its life-

cycle is completed in 7–49 days as follows: egg stage, 3–12; larval stage, 2–22; pupal stage, 2–15: it passes through 16 generations in this period. Each female can lay more than 170 eggs.

S. gracilis Mots. is a specific predator of mites and in the Punjab it has been recorded feeding on the following species: *Tetranychus cucurbitæ*, *T. tici* Hirst. *Partetranychus indicus* Hirst and *P. bioculatus*. It prefers to feed on their eggs and resting stages. In the laboratory at 33° C. adults were found to feed on, on an average, 99 eggs and the larvæ on 60–200 eggs (depending upon its age), daily.

(10) *Scymnus guimeti* Muls.—This beetle has so far been reported from Delhi and Coimbatore only: at Delhi it has been recorded feeding on Cibius red scale (*Aonidiella aurantii* Mask.).

(11) *Scymnus xerampelinus* Muls.—This beetle is common in the plains of India. Its pupal stage in the Punjab has been found to occupy about a week.

It has been recorded feeding on *Aphis gossypii* Glover (= *Aphis malvæ* Koch.) in the Punjab, and *Phenacoccus insolitus* Green in South India. Its larvæ destroy 75 aphides a day.

(12) *Scymnus* sp.—This beetle, which has been recorded from the Punjab only, remains active throughout the year. It feeds on the following Aleyrodidæ: *Trialeurodes ricini* Misra. *Dialeurodes decempunctata* Quintance and Baker, *D. citri* Ashm., and *Bemisia gossypiperda* M. and L., and on the eggs of *Pseudococcus* sp. (Coccidæ).

(13) *Sumnis renardi* Ws.—The larvæ of this beetle have often been observed at Pusa feeding on Mango mealy bug (*Monophlebus stebbingi* ver. *octocaudata* Green). They feed on the yellow coloured body fluids of its victim which they obtain by gnawing through the skin. The pupal stage of this beetle occupies 8–9 days.

(14) *Jauravia binotata* Gorh.—This beetle was found feeding in some numbers on Citrus red scale (*Aonidiella orientalis* Newst.) and Peach curl aphis (*Brachycaudus pruni* Koch.) at Lyallpur in 1939.

(15) *Chilocorus nigritus* F.—It is a widely distributed Lady-bird bettle in India. It has been reported feeding on *Pulvinaria maxima* Green in South India and aphides in the Punjab.

(16) *Ballia eucharis* Muls.—This beetle is only recorded from the Kulu valley (Punjab) where it feeds on Woolly aphis (*Eriosoma lanigerum* Haus.) as well as other aphides.

(17) *Oenopia sauzeti* Muls.—This beetle, like *B. eucharis* Muls., has so far been recorded only from the Kulu valley (Punjab) feeding on Woolly aphis and other aphids.

(18) *Cœlophora* sp.—This beetle has been reported from Nilgiris feeding on the Green bug of coffee (*Lecanium viride* Green).

(19) *Adonia variagata* s. sp. *doubledayi* Muls.—This beetle is recorded feeding on such aphids as *Macrosiphum granarium* Kirby, *Rhopalosiphum pseudobrassicæ* Davis (= *Siphocoryne indobrassicæ* Das), and *Aphis gossypii* Glover (= *Aphis malvæ* Koch.) at Lyallpur.

(20) *Rodolia* sp.—This beetle has been recorded feeding on the nymphs of *Idiocerus* spp. at Lahore.

II. Cicindelidæ.—

(21) *Cicindela sexpunctata* Fab.—This Tiger beetle is fairly widely distributed in India. It is a powerful and rapid flier. During August–September it is said to be commonly present in rice fields where it feeds on the rice bug [*Leptocorisa varicornis* F.: (Coreidæ)].

III. Carabidæ.—

(22) *Calosoma maderæ* F. var. *indicum* Hope.—This beetle is widely distributed in Northern India. Its grubs and adults which are usually found in cracks in soil and under clods, feed on the caterpillars of *Cirphis* and *Plusia* (Noctuidæ).

(23) *Calosoma olivieri* Dej.—This beetle is widely distributed in the Orient. It feeds among other insects on young locusts, *Schistocerca gregaria* Forsk. (Acrididæ), and on the larvæ of *Cirphis unipuncta* Haw. (Noctuidæ).

IV. Staphylinidæ.—

(24) *Quediosoma sericoilius* Cam.—This beetle is fairly widely distributed in India. It feeds on the young ones of *Odontoatermes obesus* Holm. (Termitidæ).

DIPTERA

V. Syrphidæ.—

(25) *Syrphus confrater* Wied.—This is a widely distributed syrphid in India. It has been reported feeding on the aphids of cotton, wheat, cabbage, chrysanthemum and mustard. In the Punjab it feeds on Woolly aphis (*Eriosoma lanigerum* Haus.) and checks its multiplication particularly during June–August and occasionally in September–October.

(26) *Syrphus balteatus* (De Geer).—This is the commonest of Indian Syrphids which is found both in the hills and plains: at Pusa it is recorded to be most abundant during January–March.

During December its life-cycle is completed in 21–27 days as follows: egg stage, 2; larval stage, 10–14; pupal stage, 9–11.

It feeds on the aphids of cotton, cabbage and maize and on the coccids infesting cotton. Depending upon its age a maggot may eat 14–57 *Aphis maidis* Fitch daily.

(27) *Syrphus albostriatus* (Fln.).—This syrphid has so far been recorded from Simla, Darjeeling, Dehra Dun and is evidently a hill species. It is recorded as feeding on aphids but what species of aphides it feeds upon is not known.

(28) *Syrphus isaaci* Bhatia.—This Hover fly has been recorded from Assam, Nepal, Mussoori, Kumaon and Pusa. It feeds on mustard aphis *Rhopolosiphum pseudobrassicæ* Davis (= *Siphocoryne indobrassicæ* Das) in February, and during this month its pupal stage occupies two weeks.

(29) *S. serarius* Wied.—This syrphid has been recorded from various hill stations in India. During February–March it feeds on mustard aphis. Its pupal stage at Pusa is said to occupy about 8 days.

(30) *Ischiodon scutellaris* Fab.—This is one of the commonest of our Syrphids which is active throughout the year. Its larvæ feed on wheat and mustard aphids in March, cabbage aphid in March–April, cotton aphid in July–September and February–March, and on aphids on water melons in May.

(31) *Sphærophoria indiana* Big.—This is a common syrphid of India which is found in the plains: It is particularly common in the western Himalayas.

Its larval stage occupies 7 days and pupal stage 4–5 days at Lyallpur. It feeds on pea aphid and on maize aphid. When 5–6 days old its maggots eat 44–63 maize aphids a day.

(32) *Paragus serratus* F.—This is a widely distributed syrphid in the Orient. Its maggots feed on aphids of red gram, water melon, *Dolichos lablab*, cotton, mustard and sugarcane.

(33) *Paragus* spp. (*P. serratus* F., *P. indicus* Brun. and *P. rufiventris* Brun.) are widely distributed in India, their larvæ feeding on aphides occurring on *Centaurea* and " Sonchus ".

NEUROPTERA

VI. *Coniopterygidæ.*—

(34) *Coniocompsa indica* Withycome.—This is a predator of very rare occurrence which has so far been recorded only from the Kulu valley feeding on the Woolly aphis (*Eriosoma lanigerum* Haus.).

VII. Chrysopidæ.—

(35) *Chrysopa scelestis* Banks.—This is a very widely distributed chrysopid in India.

It remains active throughout the year. During August–November its life-history is completed in 13–35 days as follows: egg stage, 2–5; larval stage, 6–17; pupal stage, 5–13.

C. scelestis Banks feeds on the White fly of cotton (*Bemisia gossypiperda* M. and L.); sarson aphis *Rhopalosiphum pseudobrassicæ* Davis (= *Siphocryne indobrassicæ* Das); eggs and nymphs of *Pyrilla perpusilla* Walk. and nymphs of Mango mealy bug (*Monophlebus stebbing* var. *octocaudata* Green) and citrus psylla (*Diaphorina citri* Kuw.) When food is scarce the *Chrysopa* larvæ feed upon each other.

(36) *Chrysopa* sp.—This chysopid has so far been recorded only from the Kulu valley where it is common among Woolly aphis (*Eriosoma lanigerum* Haus.) colonies during April–May.

RHYNCHOTA

VIII. Reduviidæ.—

(37) *Harpactor costalis* Stal.—This reduviid has been recorded preying upon the red cotton bug (*Dysdercus cingulatus* F.: Pyrrhocoridæ).

(38) *Nabis capsiformis* Germ.—This is a small insect which is met with abundantly in grasses. It sucks out the body juices of caterpillars.

(39) *Acanthaspis quinquespinosa* Fab.—This reduviid has so far been recorded from the Punjab only.

This bug is most active from May–October when a female lays the largest number of eggs. During this period its life-cycle is completed in 21–53 days. It feeds on tobacco aphids, larvæ of *Chilo zonellus* Swinh. (Pyralidæ) and *Laphygma exigua* Hb. (Noctuidæ) workers of Termites, grubs of *Epilachna* spp. (Coccinellidæ) and nymphs of *Diaphorina citri* Kuw. (Psyllidæ).

IX. Anthocoridæ.—

(40) *Triphleps tantilus* Mots.—This insect has so far been recorded from the Punjab only.

It feeds on cotton aphis (*Aphis gossypii* Glover) and eggs and larvæ of pink bollworm (*Platyedra gossypiella* Saund.).

X. Mantidæ.—

(41) *Humbertiella indica* Sauss.—This preying Mantis has been recorded feeding on the mango hoppers (*Idiocerus* spp.: Jassidæ) at Shalimar (Lahore) only once in 1921.

THYSANOPTERA

XI.   Thripidæ.—

   (42) *Scolothrips sexmaculatus* Pergande.—This thrips was found feeding on mites in the Punjab in 1936.

Future Lines of Research

   Insect predators occur in Orthoptera, Dermaptera, Odonata, Corrodentia, Thysanoptera, Hemiptera, Neuroptera, Mecoptera, Coleoptera, Lepidoptera, Diptera and Hymenoptera. At present, because of the paucity of information about them, it is difficult if not impossible to assess the true value and savings due to the insect predators, but as they destroy injurious insects the benefits derived by the Zamindars from them must be appreciable if not actually great. For an intelligent understanding of the influence excercised by them on the population of injurious insects of a locality it is essential that the insect predators should be collected and identified, their life-histories and habits carefully studied, and the kinds of insects on which they feed carefully noted. It should also be found out whether the insect predators breed in confinement or not, because, ultimate reinforcement of their population in nature depends upon their capacity to take advantage of the facilities offered to them by man. Observations should also be made to study those natural and biotic factors which determine the abundance or otherwise of an insect predator.

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TWO NEW SPECIES OF AVIAN TREMATODES

By B. S. Chauhan, M.Sc.

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A SINGLE specimen of a fluke of the genus *Prosthogonimus* was found in the alimentary canal of a House Sparrow, *Passer domesticus* (Linn.). The specimen is sexually mature. On examination, I found that it represents a new species of the genus. Unfortunately the description is based on only a single specimen.

Prosthogonimus macroacetabulus n. sp.

(TEXT-FIG. 1)

Body elongately oval with anterior end bluntly pointed, posterior end broad. Laterally the worm is convex on the left side and concave on the
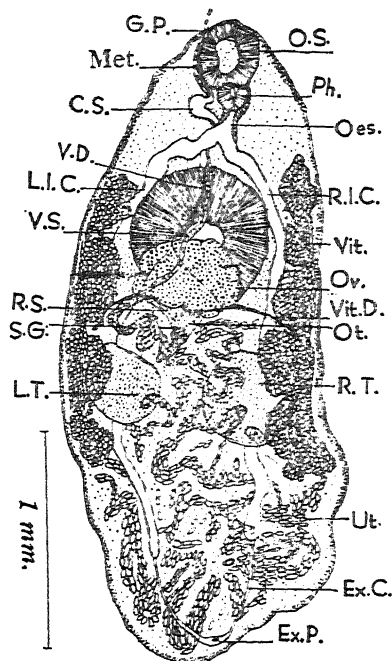
FIG. 1. *Prosthogonimus macroacetabulus* n.sp. Dorsal view

C.S., cirrus sac; *Ex. C.*, Excretary canal; *Ex.P.*, Excretary pore; *G.P.*, Genital pore; *L.I.C.*, Left intestinal cæcum; *L.T.*, Left testis; *Met.*, Metraterm; *Œs.*, Œsophagus; *O.S.*, Oral sucker; *Ot.*, Oötype; *Ov.*, Ovary; *Ph.*, Pharynx; *R.I.C.*, Right intestinal cæcum; *R.S.*, Receptaculum seminis; *R.T.*, Right testis; *S.G.*, Shell gland; *Ut.*, Uterus; *V.S.*, Ventral sucker; *Vit.*, Vitellaria; *Vit.D.*, Vitelline duct.

right. Maximum length of the body 2·94 mm. and maximum width 1·22 mm. in the posterior region of the body. Cuticular spines irregularly distributed all over the body, being more numerous at the anterior end, measuring 8–12 μ in length. Oral sucker (O.S.), more or less, oblong, subterminal and smaller than the ventral sucker. It measures 0·38 mm. by 0·272 mm. Ventral sucker (V.S.) lies immediately behind the intestinal bifurcation and touches laterally the two intestinal cæca. The distance between the point of the intestinal bifurcation and the anterior end of the acetabulum is 0·119 mm. The ventral sucker lies in the posterior portion of the anterior half of the animal and at a distance of 0·68 mm. from the anterior end of the body. It is round in shape and measures 0·64 mm. by 0·63 mm. The ratio of the oral sucker to ventral sucker is 1 : 2·34. Prepharynx absent. The oral sucker is immediately followed by a small bulbose highly muscular pharynx (Ph.) measuring 0·119 mm. in length and 0·13 mm. in width. Œsophagus (Œs.) is extremely small, hardly measuring 0·068 mm. in length. It bifurcates at a distance of approximately 0·56 mm. from the anterior end into two intestinal cæca (L.I.C. and R.I.C.), which are slightly sinuous and extend beyond the testes in the posterior region, the left one being slightly longer than the right.

Testes (R.T., L.T.) oval, slightly unequal, in the anterior portion of the posterior half of the body, partly supracæcal and post-ovarian. The vasa efferentia meet into a common duct at the level of the middle of the acetabulum, the common duct continuing in the cirrus sac (C.S.) which begins on the anterior border of the acetabulum. The left testis (L.T.) measures 0·35 mm. × 0·34 mm., the right (R.T.) 0·425 mm. × 0·40 mm. The cirrus sac is very sinuous and elongated, running to the left of the œsophagus, pharynx and the oral sucker. The metraterm (Met.) runs nearly parallel to the left of the cirrus sac and both open on the left of the oral sucker at the anterior end of the animal. The details of the coiling of the vas defferens in the cirrus sac and the nature of pars prostatica could not be made out.

Ovary (Ov.) transversely elongate, almost median, irregularly spherical and distinctly follicular measuring 0·30 mm. × 0·49 mm. A greater part of the ovary lies on the ventral posterior portion of the acetabulum. Receptaculum seminis (R.S.) and Oötype (Ot.) immediately posterior to ovary; shell gland (Mehli's gland) (S.G.) and Laurer's canal present. Vitellaria arranged in definite follicular clusters lying on the lateral sides of the body of the animal on the intestinal cæca. They are asymmetrical and begin at the level of the bifurcation of the intestine and extend beyond the testes but terminate much anteriorly than the termination of the intestinal cæca. The follicles on the left side are arranged in four groups and begin at the level

of the anterior end of acetabulum, comparatively a little posterior to those on the right side. Those on the right side are in five groups and begin at the level of the intestinal fork. Follicles on the right side also extend a little more posteriorly than those on the left side. The vitelline field partly overlaps the testes in the testicular region. The vitelline duct (Vit. D.) runs transversely at the level of the posterior border of the acetabulum. Uterus (Ut.) greatly coiled, the coils in the post-acetabular region being heavy. There are no pre-acetabular coils.

The excretary system (Ex.P.) is extremely interesting being V-shaped, not Y-shaped as in other species of the genus. The excretory pore (Ex.P.) lies sub-terminally at the posterior end.

Eggs typical of the genus. Uterine eggs (in mounted specimen) oval with posterior spine, measuring 18–26×5–$12\,\mu$.

Macy (1934*b*) gives a key to the species of the genus *Prosthogonimus* holding only 16 of the described species as valid. He excluded *P. longusmorbificans* Siefried, 1923, from his key on the ground that it was named in contravention of the international rules of nomenclature but included in his key the three species *P. skrjabini*, *P. karausiaki* and *P. horiuchii* which he considered as synonyms of some other species already included by him in his key.

Since the publication of Macy's paper, other species of the genus have been described, *viz.*, *P. leei* Hsu., 1935, *P. folliculus* Reid and Freeman, 1935, and *P. indicus* Srivastava, 1937.

I have given below a key to all the species of the genus including *P. longusmorbificans*, along with the new species described in this paper and have excluded from my key the three species *P. skrjabini*, *P. karausiaki* and *P. horiuchii* on the ground that the first two are synonymous with P. *anatinus* and the third with *P. putschkowskii*.

The original description of *P. leei* and *P. longusmorbificans* were not available to me. Therefore I have relied on such specific characters of these species as given by Macy (1934) and Srivastava (1938).

Key to the species of the genus *Prosthogonimus* Lühe, 1899.

(syn. *Prymnoprion* Looss, 1899)

1. (6) Suckers approximately of the same size 2

2. (4) (5) Cirrus pouch not extending posterior to the level of intestinal bifurcation. Vitellaria begin below the level of the intestinal bifurcation, at the level or below the level of the ventral sucker 3

3. Cirrus pouch very sinuous. Vitellaria begin in the region of ovary, anterior margin being considerably behind the level of the posterior margin of ventral sucker .... *japonicus* Braun, 1901.

Cirrus pouch not sinuous. Vitellaria begin at the level of the ventral sucker.... *pellucidus* von Linstow, 1873.

4. (2) (5) Cirrus sac extending past the intestinal bifurcation but not reaching the acetabulum. Vitellaria begin at the level of the intestinal bifurcation...... *folliculus* Reid and Freeman, 1935.

5. (2) (4) Cirrus pouch reaching nearly to acetabulum. Vitellaria begin below the level of intestinal bifurcation, at the anterior border of the ventral sucker.... *furcifer* Railliet, 1924.

6. (1) Ventral sucker at least a half larger than the oral sucker   7

7. (15) Ovary dorsal to ventral sucker or at least considerably overlapping it   8

8. (10). Uterus with heavy pre-acetablur coils   9

9. Vitellaria extending much posterior to testes.... *ovatus* Rudolphi, 1803 (Type species).

Vitellaria not reaching the posterior margin of the tests.. *dogiele* Skrjabin, 1914.

10. (8). Uterus without heavy pre-acetabulur coils   11

11. (13). Cirrus sac reaching the acetabulum, œsophagus extremely small   12

12. Vitellaria restricted, post-acetabular.. *leei*. Hsu., 1935.

Vitellaria not restricted to post-acetabular region.. *macro-acetabulus* n. sp.

13. (11). Cirrus sac extending below intestinal bifurcation but not reaching to acetabulum   14

14. Testes and vitellaria confined to anterior half of body.. *vitellatus* Nicoll, 1915.

Testes not in the anterior region. Vitellaria not confined to anterior half.. *indicus* Srivastava, 1937.

15. (7) Ovary definitely posterior to ventral sucker   16

16. (20) Cirrus pouch reaching ventral sucker 17

17. (18) Ventral sucker situated immediately after the intestinal fork ..*brauni* Skrjabin, 1919.

18. (17) Ventral sucker situated at some distance posterior to the intestinal fork 19

19. Sucker ratio nearly 1: 1·5..*longusmorbificans* Siefried, 1923
    Sucker ratio 1: 1·23..*putschkovskii* Skrjabin, 1913.

20. (16) Cirrus pouch not reaching ventral sucker 21

21. (22) Anterior border of vitellaria definitely post-acetabular. Testes located at the middle of the longitudinal body axis ..*anatinus* Markow, 1902.

22. (21) Vitellaria begin at the level of the acetabulum. Testes post ovarial 23

23. (24) Vitellaria ending at or before the posterior margins of testes..*macrorchis* Macy, 1934.

24. (23) Vitellaria extend much posterior to testes 25

25. (26) Coils of the uterus not reaching lateral portions of the body, entirely inter-cæcal..*rudolphii* Skrjabin, 1919.

26. (25) Uterine coils extending laterally over the intestinal cæca and filling most of the posterior half of body .. 27

27. Cirrus sac reaching intestinal fork, nearly straight: ratio between the oval and ventral sucker as 1: 2..*cuneatus*. (Rudolphi, 1809).

    Cirrus pouch extends farther, very sinuous: ratio between the oral and ventral suckers as 1: 3..*fuelleborni* Skrjabin and Massino, 1925.

2. *Eumegacetes microdiosus* n. sp.

(Text-Fig. 2)

A single specimen of this fluke belonging to the genus *Eumegacetes* Looss, 1900, was found in the alimentary canal of black-headed Maina, *Temenuchus pagodarum* (Gm.). The specimen is sexually mature. A study of the worm reveals that it is a new species of the genus.

Body elongately oval with rather blunt anterior end and slightly pointed posterior one. Maximum length of the body is 3·35 mm. and maximum width 1·66 mm. in the region of the acetabulum. Suckers large.
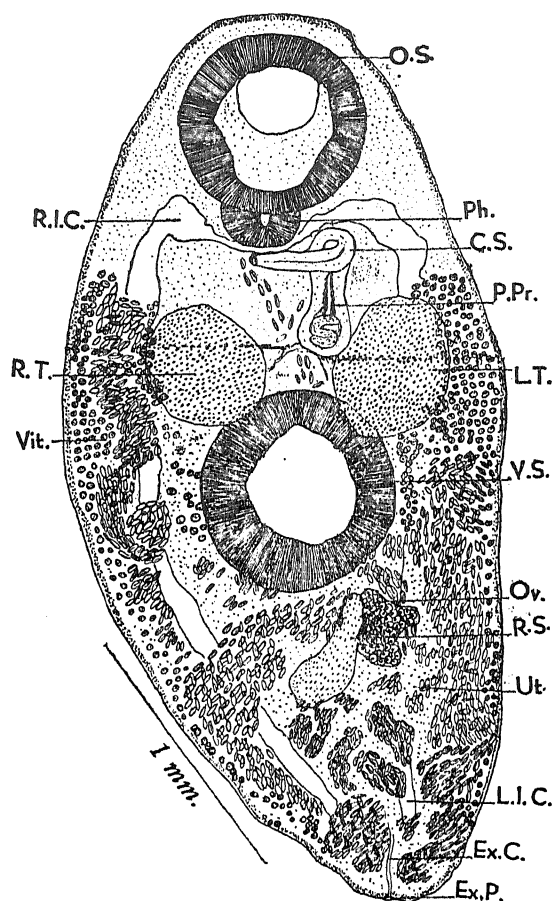
FIG. 2. *Eumegacetes microdiosus* n. sp., Dorsal view.

P.Pr., Pars prostatica; Other lettering as in Fig. 1.

Oral sucker (O.S.) is entirely sub-terminal, being situated at a distance of 0·08 mm. from the anterior end of the animal and measures 0·66 mm. × 0·67 mm. The posterior sucker (V.S.) slightly posterior to the middle of the body length and lies much posterior to the intestinal bifurcation and measures 0·74 mm. × 0·70 mm. The ratio of the ventral sucker to the oral sucker is 1 : 0·95. The ventral sucker lies at a distance of 1·40 mm. from the anterior end. Prepharynx absent. Pharynx (Ph.) strong, semi-circular, measuring 0·12 mm. by 0·29 mm. Œsophagus entirely absent. The intestinal cæca (L.I.C. and R.I.C.) are long and are at first transversely disposed and later bend to run in the usual manner nearly to the posterior end of the body.

Testes (R.T. and L.T.) are spherical, preovarian, inter-cæcal and are almost symmetrically situated. The left testis is slightly bigger and more elongated than the right one measuring 0·50 mm. × 0·43 mm. The right testis (R.T.) measures 0·45 mm. × 0·43 mm. The testes, particularly the left one, are slightly supra-acetabular. The two vasa efferentia are distinct, the right one seems to be slightly longer than the left. Cirrus sac (C.S.) is very long and sinuous lying close to the left. The approximate length of the cirrus sac is 1·18 mm. and maximum width in the basal region 0·16 mm. It is saccular at the base. The cirrus sac just below the intestinal cæcum on the left side bends on itself and runs transversely to the body to open immediately behind the pharynx. Pars prostatica (P.Pr.) present.

Ovary (Ov.) irregularly shaped, post-acetabular, inter-cæcal, situated slightly to the left side. It is longer than broad. Shell gland (S.G.) present. Receptaculum seminis (R.S.) is pear-shaped lying on the right side of the ovary. Its wider portion is directed posteriorly and the pointed end anteriorly. Uterine coils (Ut.) very heavy, practically overlapping the whole intestinal cæca and partly vitellaria also. The genital pore opens at the level of the posterior end of the pharynx slightly to the right side. Vitellaria (Vit.) are symmetrical and begin in the pre-testicular zone, overlap the intestinal cæca and terminate a little distance beyond the hinder ends of the intestinal cæca. Excretory system Y-shaped with a short stem. Eggs pointed, measuring $29 \times 13\,\mu$.

Discussion

Mehra (1935) gives a key to the species of the genus *Eumegacetes* Looss, 1900.

The species described in this paper differs from E. *contribulans* Braun, 1901, E. *medioximus* Braun, 1901 and E. *braunii* Mehra, 1935, in the extent of the vitellaria, which in this species extend anterior to testes. The new species also differs from E. *braunii* in the position of the ovary which is situated much anterior to the termination of the intestinal cæca. The present species resembles E. *perodiosus* Travassos, 1922 in the extent of vitellaria. It, however, differs from it in having the ventral sucker larger than the oral sucker.

E. *microdiosus* n. sp. further differs from E. *perodiosus* in the fact that the testes are pre-equatorial, though it resembles E. *emendatus* Braun, 1900 and E. *artamii* Mehra, 1935 in this respect. It differs from E. *artamii* in having the acetabulum unequal and from E. *emendatus* in the cirrus sac being not U-shaped. Unlike E. *artamii*, the testes are partly supra-acetabular in the new species.

B. S. Chauhan

Eumegacetes microdiosus n. sp.

Specific diagnosis.—*Eumegacetes* Looss, 1900 syn. *Megacetes* Looss, 1899.

With generic characters. Body elongately oval, anterior end blunt, posterior end pointed. measuring 3·35 mm. × 1·66 mm. Oral sucker sub-terminal 0·66 mm. × 0·67 mm. Posterior sucker partly equatorial 0·74 mm. × 0·70 mm. Sucker ratio of the ventral sucker to the oral 1 : 0·95. Pharynx semicircular 0·12 mm. × 0·29 mm. Testes spherical, pre-equatorial, partly supra-acetabular. The right one is smaller than the left, measuring 0·45 mm. × 0·43 mm. The left measures 0·50 mm. × 0·43 mm. Cirrus sac long. sinuous and coiled upon itself to form a loop and afterwards runs transversely. Ovary posterior to acetabulum. Receptaculum seminis pear-shaped with the broad end posteriorly directed. Vitellaria begin in the pre-equatorial region and extend much posteriorly. Excretory system Y-shaped. Eggs pointed measuring (maximum) 29 × 13 μ.

Host: Black-headed Maina (*Temenuchus pagodarum*) (Gm.)

Location: Alimentary canal.

Locality: Nagpur, C.P., India.

In conclusion, I am grateful to Dr. M. A. Moghe, Head of the Department of Zoology, for his keen and personal interest in me. I also wish to express my thanks to Principal Dr. K. Krishnamurti for giving me the necessary facilities for work.

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THE ARTERIAL SYSTEM OF THE POND-TURTLE, *LISSEMYS PUNCTATA* (BONNATERRE)

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Introduction

THE importance of the pond-turtle *Lissemys punctata* (Bonnaterre) as a Testudinate type for class dissections has already been emphasized by Mathur (1940), who has given a detailed description of the venous system of this species. The present paper, dealing with the arterial system, is in continuation of Mathur's work and will be followed by another on the anatomy of the heart.

As far as I can ascertain, we are indebted to our earliest knowledge of the arterial system of Turtles to Bojanus (1819–21), who gave a careful account of the anatomy of *Emys europaea*. Fritsch (1869), in his comparative account of the hearts of Amphibia and Reptilia, dealt with several species of turtles. Sabatier (1873, 1874)* gave a general description of this system. Pitzorno (1905) dealt with the comparative morphology of the subclavian and axillary arteries. Burne (1905) made a few concise remarks on the arterial trunks of the leathery turtle *Dermochelys coriacea*. O'Donoghue (1917) brought together the relevant facts about the occurrence of the ductus arteriosus. Kimball (1923) studied the anatomy and development of the arterial and venous systems. Bremer (1924) made an investigation of the factors governing the position of the cœliac artery in turtles as compared to that in the chick. Koch (1934) described a thickening (*Sphincter Arteriæ pulmonalis*) in the wall of the pulmonary artery in *Chelone midas*, *Caretta caretta* and *Dermochelys*, while Atwood (1923) and Hyman (1939) gave generalised accounts of the vascular system of turtles for use in class dissections.

Material and Technique

Over two dozen specimens, caught at Runkuta, Malpura, Sikandra Rau and Jalesar (Agra District), were employed for the present study. They varied in length of carapace from 5 to 10 inches and included both sexes.

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* Hoffmann's account (1890) of the arterial system of Turtles is based on Sabatier's work.

84

Three injection masses were used:

(*a*) *Davison's Starch Mass,* prepared as follows:—
>   ·100 c.c. water
>   20 c.c. glycerin
>   20 c.c. concentrated formalin
>   85 grams corn starch
>   sufficient vermilion to give a deep colour.

(The whole mixture is to be stirred thoroughly and strained through a fine muslin-cloth).

(*b*) *Emery's Aqueous Carmine,* prepared (as recommended by Bhatia and Dayal, 1933) by adding dilute acetic acid to a 10 per cent. ammoniacal solution of carmine until the colour of the solution changes to blood-red. " The supernatant clear solution is injected cold without further preparation. The injected organs are thrown at once into strong alcohol to fix the carmine."

(*c*) *Reeve's Fixed Indian Ink,* injected into individual arterial trunks without any further preparation. This fluid, suggested to me by Mr. Mahendra, has served as an invaluable help in tracing the finer branches of the arteries and can be strongly recommended.

The Origin of the Arterial Arches

Three separate arches (Text-Fig. 1) arise independently from the anterolateral ventral border of the ventricle: the *Pulmonary* Arch,   the
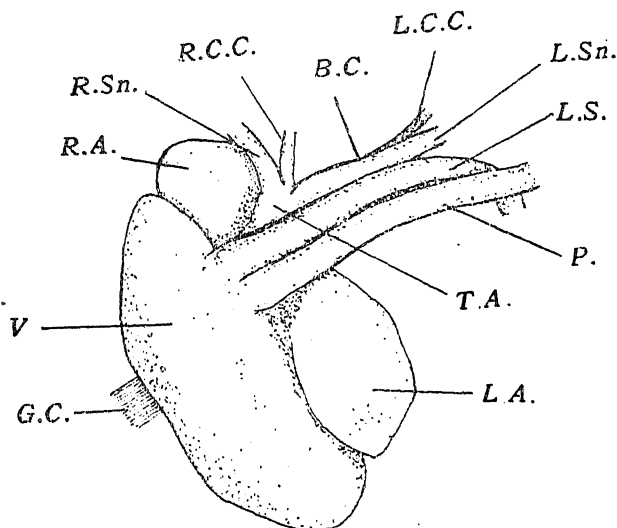
TEXT-FIG. 1.   The Heart of *Lissemys punctata*

B.C., left brachio-cephalic artery; *G.C.,* gubernaculum cordis; *L.A.,* left auricle; *L.C.C.,* left common carotid artery; *L.S.,* left systemic arch; *L.Sn.,* left subclavian artery; *P.,* pulmonary artery; *R.A.,* right auricle; *R.C.C.,* right common carotid artery; *R.Sn.,* right subclavian artery; *T.A.,* Truncus anonymus; *V.,* ventricle.

B3*a*

F

Left Systemic Arch and the *Right Systemic (Carotico-systemic)* Arch. They are bound together at their base by connective tissue and originate from the left side of the ventricle.

The *Pulmonary* and the *Left Systemic* Arches lie side by side on the ventral aspect of the heart, extending forwards obliquely towards the left. The *Pulmonary* Arch, soon after its origin, divides into the right and the left pulmonary arteries. The *Left Systemic* Arch lies to the right of the Pulmonary Arch, runs towards the left parallel to it and finally crosses over dorsal to the left pulmonary artery to sweep backwards and inwards to meet the Right Systemic Arch.

The *Right Systemic* Arch arises dorsal to the Left Systemic and is, therefore, scarcely visible in a ventral view of the heart. It runs almost straight forwards, giving off a large trunk, the *Truncus anonymus* (the *Innominate* Artery), at its anterior aspect and then turns upwards and backwards to meet the Left Systemic.

The Truncus Anonymus and its Branches

The *Truncus anonymus* or *Innominate* Artery (Text-Fig. 1), immediately after its origin from the Right Systemic Arch, forks into two large branches, the *brachio-cephalic* arteries, each of which soon divides into an inner and an outer artery. The inner artery is the *common carotid (Arteria carotis communis)*, while the outer one is the *subclavian (Arteria subclavia)*.

It is interesting to note that the left carotid and subclavian arteries are distinctly longer than the right ones, the disparity in size being apparently correlated with the position of the heart not in the median line but remarkably to the right of the base of the neck. Such a position of the heart is perhaps the result of the retractility of the neck and tends to develop an asymmetry in the anterior arteries by lengthening out the left ones. Curiously enough, this asymmetric position of the heart has not been previously noted. Hoffmann's diagram of the viscera *in situ* of *Clemmys terrapin* (Taf. XXXIX, Fig. 1) portrays a mesially-situated heart, while the figure of the arterial system of *Emys europaea*, given by Wiedersheim and Parker (1907) shows an almost symmetric arrangement of the carotid and subclavian arteries. I hope to investigate this asymmetry and its development in greater detail later on.

The left *common carotid* artery (Text-Fig. 2), after taking its origin from the left brachio-cephalic stem, runs forwards and outwards, crossing the base of the neck obliquely from the right side to the left and then runs
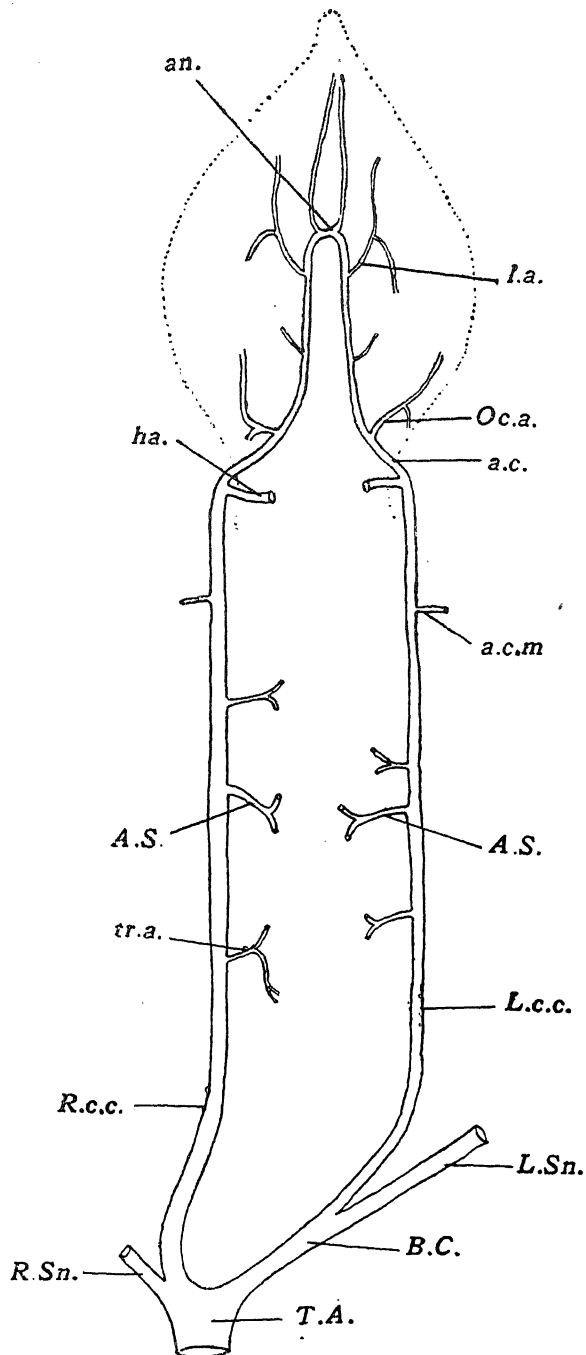
TEXT-FIG. 2.   The Common Carotid Arteries and their Branches

a.c., Arteria carotis; *a.c.m.,* cervical muscular artery; *an.,* anastomosis; *A.S.,* Arteria spinalis; *ha.,* Arteria hyoidea; *l.a.,* lateral branch from Arteria carotis; *Oc.a.,* occipital artery; *tr.a.,* tracheal artery (other abbreviations as in Fig. 1).

straight cephalad parallel and adjacent to the vagus nerve. The right *common carotid* artery, on the other hand, does not pass across the neck, but extends almost directly forwards from its point of origin along the right side.

Each common carotid artery, during its passage in the neck, sends several minute branches to the trachea and the cervical muscles, besides giving off a fair-sized artery (*Ramus anastomoticus* or *Arteria spinalis*) to the vertebral column. This latter artery soon divides into an anterior and a posterior branch, entering into the spinal canal through the third and the fourth inter-vertebral foramina respectively. As described by Hoffmann (1890), the Ramus anatomoticus enters the spinal canal only through the fourth cervical intervertebral foramen.

On reaching the posterior region of the head, the common carotid artery divides into two branches: (*i*) an *Arteria hyoidea*, supplying the tongue and the muscles of the hyoid, and (*ii*) an *Arteria carotis*, extending forwards on the base of the skull and supplying the various regions of the head.

The *Arteria carotis* extends inwards, giving off a branch to the muscles behind the occipital region, and enters the skull through the carotid canal of the pterygoid. It runs forwards on the ventral aspect of the brain and is connected to its fellow of the other side by a transverse *anastomosis* behind the hypophysis. A little posterior to this anastomosis it gives off a lateral artery that comes out of the cranium and supplies the orbit and the adjacent regions.

The *Left Subclavian artery* (Text-Fig. 3) runs from its place of origin from the brachio-cephalic stem, outwards and forwards, giving off a small
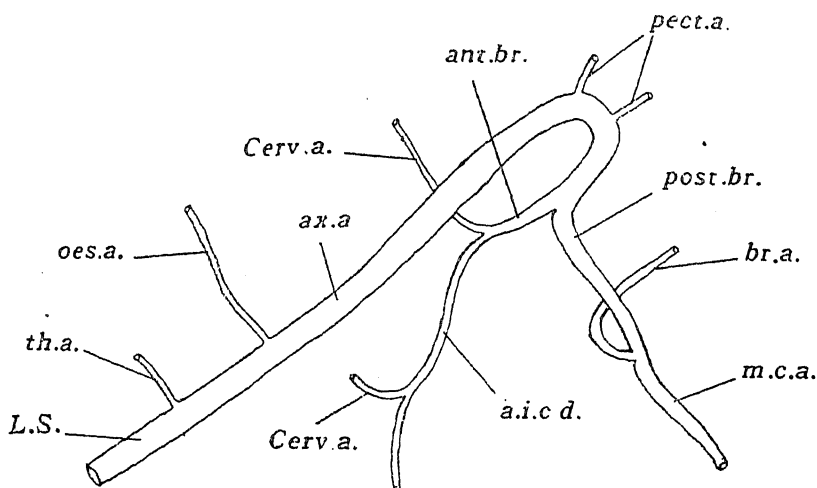
TEXT-FIG. 3. The Left Subclavian Artery and its Branches

a.i.c.d., Arteria intercostalis communis descendens; *ant.br.*, anterior branch of the axillary artery; *ax.a.*, axillary artery; *br.a.*, brachial artery; *Cerv.a.*, cervical artery; *L.S.*, left subclavian artery; *m.c.a.*, margino-costal artery; *œs.a.*, Ramus œsophageus; *pect.a.*, pectoral arteries; *post.br.*, posterior branch of the axillary artery; *th.a.*, thyroidean artery.

branch to the thyroid (*Arteria thyreoidea* or *Bojanus's Arteria thymica*) and then a larger one to the œsophagus (*Ramus œsophageus*).   The *Right Subclavian* is similar to the left, but is much shorter and gives rise to no œsophageal artery.   After giving off these branches, the subclavian continues outwards as the *Axillary Artery* (*Arteria axillaris*) and gives off two branches to the muscles of the pectoral girdle (*Mm. testo-coracoideus, subscapularis*, etc.).   As described by Hoffmann (1890), these muscles are supplied by a branch of the *Arteria brachialis*—a condition different from what I find in *Lissemys punctata*.   The axillary artery divides distally into the following two branches:

(i) An *Anterior branch*, which bifurcates into a *cervical* artery going to the neck muscles, and a *longitudinal costal* artery, running backwards dorsal to the proximal ends of the ribs, where the latter abut the vertebræ.   The latter corresponds to the *Arteria intercostalis communis descendens* of Hoffmann (1890) and runs along in the canal bounded above by the plates of the carapace and below by the bases of the ribs.   Before its entry into this longitudinal canal, it sends a small artery to the posterior part of the neck.   It supplies the shell (*vide infra*).

(ii) A *Posterior branch*, running backwards and giving off a large vessel (the *Brachial Artery*) into the fore-limb and another (the *margino-costal artery*) to the shell.   The latter extends backwards along the inner margin of the shell between the carapace and the plastron and sends transverse vessels to supply these structures.

The Systemic Arches and the Dorsal Aorta

The *Left Systemic Arch*, as already mentioned, takes its origin to the right of the pulmonary artery and runs forwards parallel to it, turning towards the left side and crossing the left pulmonary artery (Text-Fig. 4).   Then it sweeps upwards, backwards and inwards towards the place of its union with the right systemic. Before uniting its fellow of the other side, it gives rise to the following branches:

(i) A *Gastric Artery*, supplying the œsophagus and stomach and sending a minute vessel to the liver.

(ii) A *Mesenteric Artery*, arising at the point of origin of the gastric artery, but running backwards to supply the small intestine.

(iii) A *Hepato-intestinal Artery*, arising posterior to the mesenteric artery and supplying the intestine and the liver.

The *Right Systemic Arch* runs forwards from its origin dorsal to that of the Left, and after giving rise to the *Truncus anonymus*, immediately turns
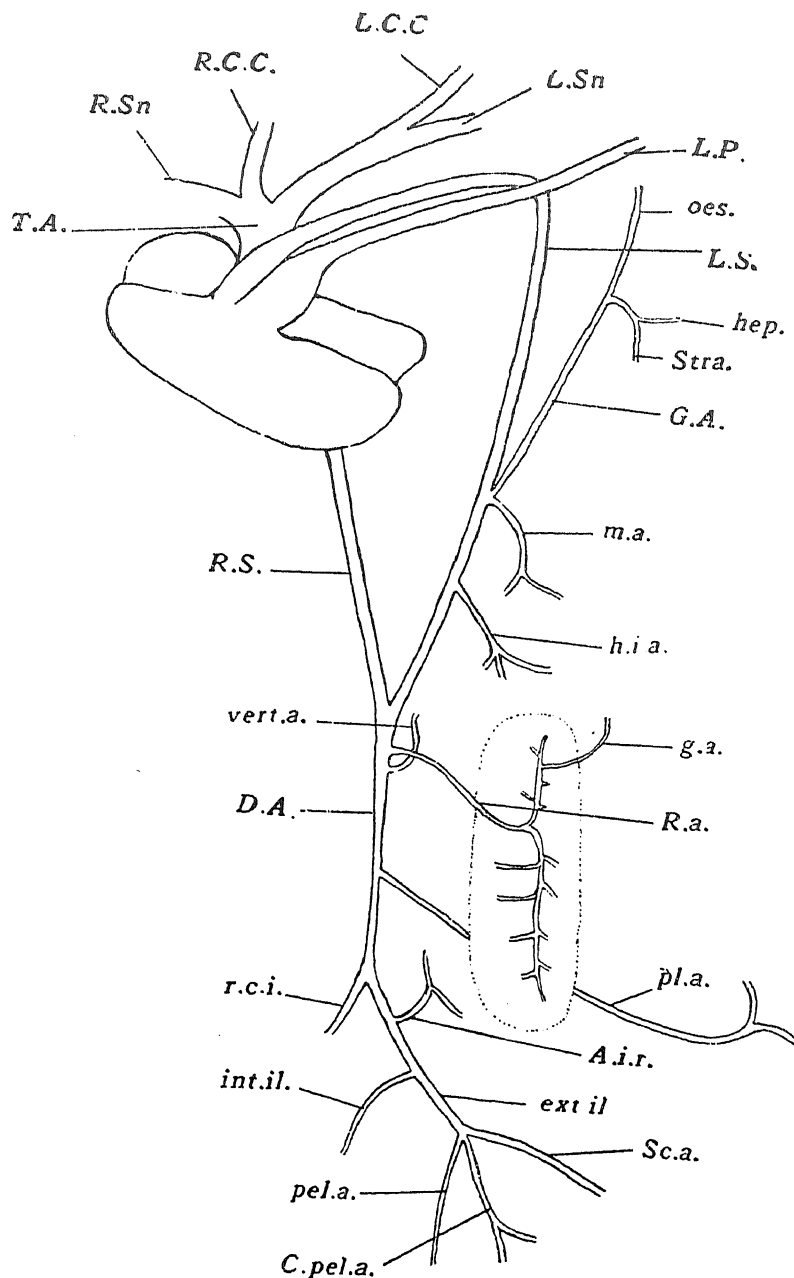
TEXT-FIG. 4. The Systemic Arches and the Dorsal Aorta

a.i.r., Arteria intercostalis recurrens; *c.pel.a.*, caudo-pelvic artery; *D.A.*, dorsal aorta; *ext.il.*, external iliac artery; *g.a.*, genital artery; *G.A.*, gastric artery; *hep.*, hepatic artery; *h.i.a.*, hepato-intestinal artery; *int.il.*, internal iliac artery; *L.P.*, left pulmonary artery; *m.a.*, mesenteric artery; *oes.*, œsophageal artery; *pel.a.*, pelvic artery; *pl.a.*, plastral artery; *R.a.*, renal artery; *r.c.i.*, right common iliac artery; *R.S.*, right systemic arch; *sc.a.*, sciatic artery; *st.a.*, artery to stomach; *vert.a.*, vertebral artery (other abbreviations as in previous figures).

upwards, backwards and slightly inwards to meet the left systemic. It gives rise to a small *coronary* artery near the origin of the Truncus anonymus, supplying the wall of the ventricle.

According to Hoffmann (1890), the Left Systemic Arch in Turtles continues mainly as the *cœliac* artery, being connected to the Right Systemic by a more or less transverse anastomosis (*Ramus anastomoticus*) of variable calibre. In *Lissemys punctata*, the condition of aortic union is remarkably different in two ways: In the first place, the two systemic arches meet together in a V-shaped manner, there being nothing corresponding to an anastomosis. Secondly, the place of union is remarkably posterior, being behind the level of the anterior borders of the kidneys. This unique condition results in a considerable shortening of the dorsal aorta.

The *dorsal aorta* runs backwards in the mid-dorsal line between the two kidneys. It gives rise, just behind its formation, to a pair of *renal* arteries, which also supply the gonads, and from almost the same place, a pair of *vertebral* arteries, going inside the vertebral column. Some distance behind the origin of these arteries, it gives a pair of branches, passing dorsal to the kidneys and supplying the plastron. It bifurcates behind to form the right and the left *common iliac* arteries, and unlike the condition mentioned by Hoffmann (1890) and others, is not continued posteriorly to form the *Arteria caudalis*.

Each *common iliac* artery first gives off a branch (*Arteria inter-costalis recurrens*) and then divides into an *internal iliac* and an *external iliac* artery.

The *Arteria inter-costalis recurrens*, after running forwards and upwards, divides into an anterior branch, running lateral to the vertebral column to unite with the *Arteria intercostalis communis descendens*, and a posterior branch (*Art. coccygea superior*) going to the post-sacral region of the vertebral column.

The *internal iliac* artery supplies the rectum, cloaca and the urinary bladder.

The *external iliac* artery divides into three branches: one going to the pelvic muscles; the second, besides supplying the pelvic muscles, sending a branch to the caudal muscles; and the third supplying the hind-limb.

The Arteries of the Shell

The carapace (Text-Fig. 5) is supplied on each side by two longitudinal arteries: The *Arteria intercostalis communis descendens* (with which is united the *Arteria intercostalis recurrens* branch of the common iliac) and the *Margino-costal* artery. Both these are branches of the axillary artery. They
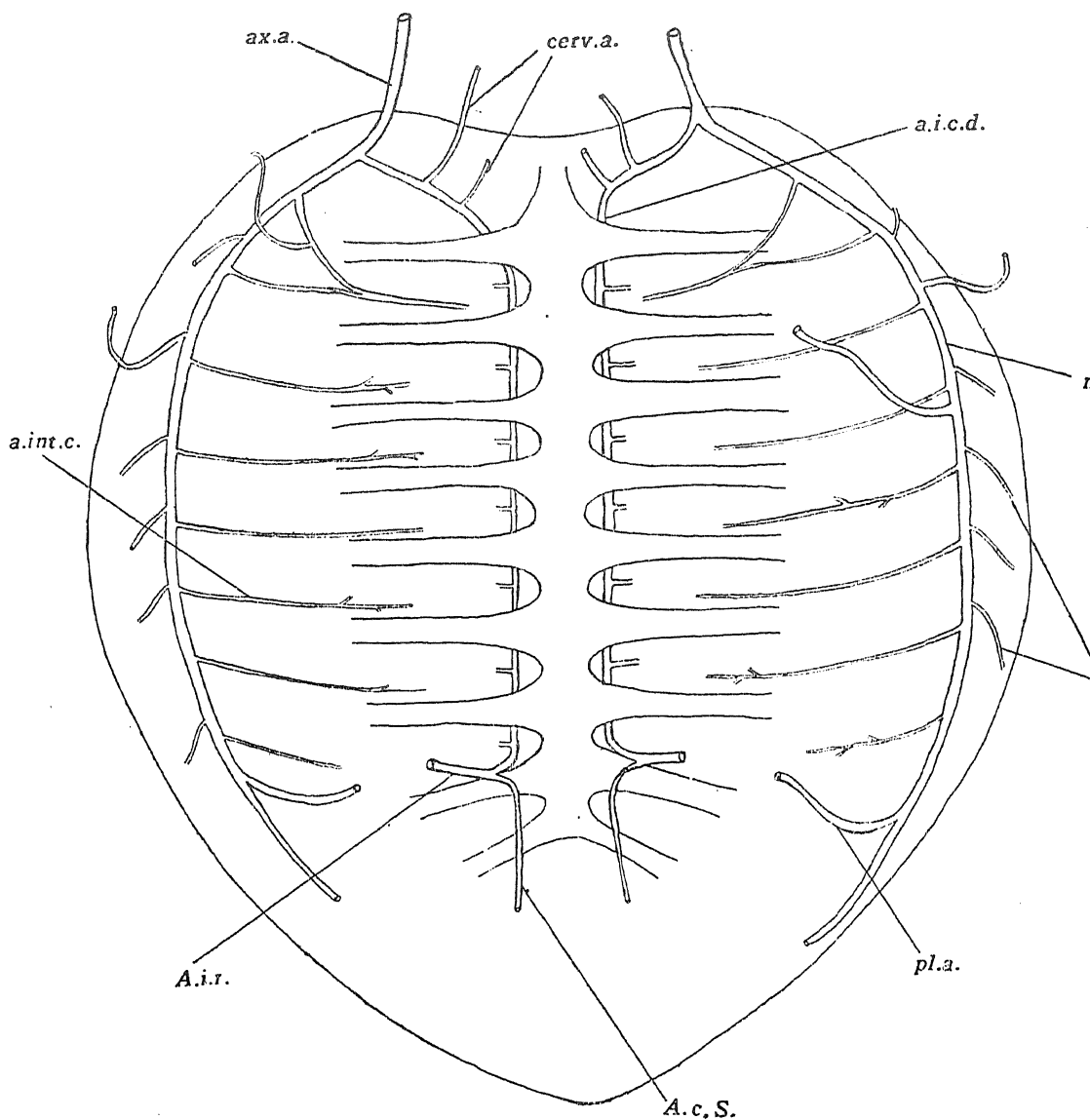
TEXT-FIG. 5.   The Arteries of the Shell

A.c.S., Arteria coccygea superior; a.i.c.d., Arteria intercostalis communis descendens; A.i.r., Arteria intercostalis recurrens; a.int.c., Arteria intercostalis; ax.a, axillary artery; cerv.a., cervical arteries; m.a., marginal branches of the margino-costal artery; m.c.a., margino-costal artery; pl.a., plastral artery.

send transverse vessels between the ribs, the former sending them outwards and the latter inwards.   Besides these branches (Arteriæ inter-costales), the Arteria intercostalis communis sends a series of inter-vertebral arteries into the vertebral column,   while   the   margino-costal artery (also called the

Arteria mammaria recurrens interna) supplies branches to the plastron and the skin contiguous to the bases of the fore- and hind-limbs. The latter artery unlike the condition described by Hoffmann, does not open posteriorly into the Arteria iliaca.

The plastron, as already mentioned, also receives its blood supply from a pair of branches from the dorsal aorta.

The Pulmonary Arteries

The *Pulmonary Arch*, shortly after its origin from the ventricle, divides into the right and the left *Pulmonary arteries*.

The Left *Pulmonary Artery*, which is the longer of the two, runs across ventral to the neck in close company with the left systemic trunk, with which it is connected by an obliquely situated anastomosis, the *ductus arteriosus*. As O'Donoghue (1917) points out, the ductus caroticus is normally absent in Chelonia, while the ductus arteriosus is represented on both sides. The diameter of the pulmonary artery diminishes to some extent distal to the point of origin of the ductus arteriosus, and some distance after this point, the artery in question crosses the systemic arch and enters the inner border of the lung just behind the latter's anterior end close to the entrance of the bronchus. It ramifies inside the lung.

The *Right Pulmonary* Artery is similar to the left, although much shorter in extent. It is also connected to the right systemic arch by a *ductus arteriosus*.

Summary

The author gives a detailed description of the arterial system of the Pond-Turtle *Lissemys punctata*. The following are the more important new features discovered by him:

(1) The left carotid and subclavian arteries are much longer than the right ones, the asymmetry being probably due to the position of the heart to the right of the base of the neck.

(2) The *Ramus anastomoticus (Arteria spinalis)* of the common carotid artery enters the spinal canal through two inter-vertebral foramina, and not through one, as described in other turtles.

(3) The muscles of the pectoral arch are supplied by a branch of the *axillary artery*, and not of the brachial artery.

(4) The *cœliac artery* is represented by three separate branches of the left systemic trunk.

(5) The *aortic union* is not formed by a transverse anastomosis, but by the actual coming together of the two systemics. It is situated very much posteriorly, the dorsal aorta being, consequently, extremely abbreviated.

(6) The *dorsal aorta* is not prolonged behind to form the caudal artery.

(7) The *dorsal aorta* gives rise to a pair of *renal* arteries, a pair of *vertebral* arteries, and a pair of *plastral* arteries.

(8) The *Arteria mammaria recurrens interna* does not open posteriorly into the iliac artery.

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STUDIES IN THE DISEASES OF *MANGIFERA INDICA* LINN.

II. Effect of Injecting Healthy Mango Fruits with Extract from Naturally Occurring Necrotic Mangoes

BY S. N. DAS GUPTA AND G. S. VERMA

(From the Department of Botany, Lucknow University)

Introduction

IN a previous paper of this series an account has been given of the external symptoms of the necrosis of the mango fruit. The disease which is very prevalent in Bihar and the United Provinces first appears as little ætiolation at the distal end of the fruit. The ætiolated area increases in size and intensity and is followed by the appearance of greyish brown spots which coalesce to form a continuous necrotic area. The disease advances and considerable part of the mango fruit is destroyed. The paper also dealt with a few preliminary experiments that had been carried out to find out the organisms responsible for the disease. Neither a fungus, nor a bacterium could be isolated from the diseased tissue and attempts to find out if the disease could be transmitted by inoculating a small piece of the necrotic tissue into the healthy mango fruit proved inconclusive. The method adopted for the latter experiment was the one developed by Murphy and M'Kay (1926) to infect the potato tubers with virus. It consisted in removing a small plug of healthy tissue by means of a cork-borer and replacing it by a plug removed from the diseased part by means of another cork-borer of next larger size.

In order to find out the immediate cause of the disease a systematic investigation was started at this laboratory along various lines. The experiments were designed to produce necrosis by subjecting healthy mango fruits to coal gas, sulphur dioxide, carbon monoxide, etc., and by injecting the extract from the necrotic tissue into the healthy fruit.

The present paper deals exclusively with the effect of injecting healthy mango fruits with extract from naturally occurring necrotic mangoes.

Method and Material

The investigation on the effect of injecting the healthy mango fruits with extract from the naturally occurring necrotic mango involved, firstly

the preparation of the extract in complete aseptic conditions and secondly evolving a method of injecting fruits so that the fruits could retain the fluid extract for a relatively longer period.

(a) *Preparation of Extract.*—Due to the lack of facilities in the department, the preparation of the extract was carried out at the Provincial Hygiene Institute, Lucknow.

Usually about twenty-five mangoes showing advanced stage of necrosis were utilised. The mangoes were first wiped with rectified spirit and the diseased tips separated from the healthy portion with a sterilised scalpel. These tips were crushed in a sterilised juice extractor with 200 c.c. of normal saline. The extract so obtained was filtered through Berkfeld's filters with the help of an electric vacuum pump at 10 to 15 mm. pressure. The filtrate was first tested for sterility, by inoculating it into a suitable nutritive medium. It was then filled in 1 c.c. ampoules and sealed in vacuum. Prior to the injection experiment, the sterility of the extract was again tested by similar method.

The extract from healthy tissue was prepared and sealed in 1 c.c. ampoules in the same manner. In this case, however, particular care was taken to collect the fruit samples from localities and trees where necrosis was unknown.

(b) *Method of Injection.*—The injection experiments were carried out in orchards with mangoes still on trees.

The fruits were first wiped with cotton wool dipped in rectified spirit to effect surface sterilisation.

Earlier attempts to inject the fruits with fluids directly by means of a syringe had shown that not only is it difficult for the needle to penetrate the mango tissue, but also the needle becomes completely blocked up by the tissue with the result that it is impossible to discharge the fluid into the tissue at all. It was therefore necessary first of all to bore a passage by means of a needle in order to inject the extract in the cavity thus formed. For this purpose, the sterilised mango fruit was pierced at a given point by means of a sterile needle (diameter 0·8 mm.). It was, subsequently found convenient to have two such passages intersecting each other at right angles. The first passage AA' was made at right angles to the long axis of the first and the second passage BB' was made perpendicular to AA' and parallel to the long axis intersected each other so that the injection of extract at one end A enabled the extract to permeate the whole passage and reach the interior of the tissue at B'.
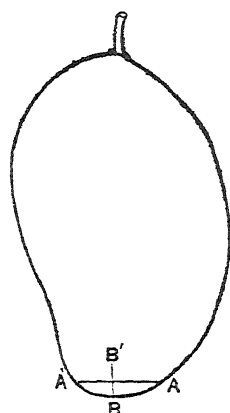
TEXT-FIG. 1

Diagrammatic representation of the two passages AA′, and BB′, intersecting each other

After the passages had been made the extract from one of the ampoules was drawn in, in a sterilised syringe and injected at the point A (Text-fig. 1) and forced into the tissue till the fluid flowed out the two free ends A′ and B (Text-fig. 1).

It was not required to seal the external punctures as it was found that in mangoes under investigation the gum appearing from the injured tissue due to the puncture effectively closed all ends which are in open communication with the atmosphere namely, A, A′ and B (Text-fig. 1). In fact, although in 1939 alone 407 mangoes have been inoculated by this method during the course of the experiment there was not a single case of rot due to foreign infection.

Mangoes given blank injections and those injected with normal saline, and with extract from healthy mangoes separately were used as controls.

The injected mangoes on the trees were kept under observation and every change from normal condition was observed and noted.

An attempt was made to make the injection experiments with mangoes of various stages of development, starting from those which had just set to those showing advanced state of maturity. But as the first symptom of necrosis appears at a definite stage of maturity of the fruit and cannot be detected at a very early stage it was not possible to inject the mangoes with fresh extract before the mangoes had attained relatively big size. To avoid this difficulty the extract of the necrotic mangoes of one season kept in a refrigerator running at a temperature of 10° C. to 15° C. was utilised for

B1*a*

injecting a mango crop of the following season. The extract of the previous season did not show any bacterial growth on test before or after the inoculation experiment had been completed. In spite of the fact that old extract was utilised for injection and the mangoes used for inoculation were relatively well developed, the results obtained were very satisfactory.

As preliminary investigation of the previous season had shown that better results were obtained with fluids injected at the distal end (Text-fig. 1) than injection at any other part of the fruit, in all subsequent experiments only the distal end was injected.

Only healthy Dasehri and Safeda (Lucknow) varieties were utilised for injection experiment, as these important varieties are being heavily damaged by the necrosis. The bulk of the experimental work was carried out with Safeda mangoes as a larger number of Safeda trees were available for investigation and these had a far heavier crop than the Dasehri trees.

The extract used for injection was from necrotic Dasehri and Safeda collected from Bahadurpur orchard.

The injection experiment was carried out at the Government Horticultural Garden, Lucknow, and in a private orchard belonging to Mr. D. N. Sanyal. These orchards, situated within the Municipal area of Lucknow do not suffer from the necrosis.

Effect of Injection.—A number of healthy Dasehri and Safeda Mangoes were injected with extract from diseased tissue. Two different extracts were utilised ; one prepared and ampouled towards the end of the previous (1938) mango season (*old extract*) and the other prepared and ampouled in the current 1939 season (*fresh extract*). Preliminary experiments were done in 1938 mango season and as certain interesting results were obtained, the work was extended and elaborated in 1939 mango season. The results obtained in 1939 using both the old and fresh extract and the results of the control experiments are given in the tabular form.

In the tables in the third column is given the number of mangoes actually injected. Under the wastage column (fourth column) are included those mangoes which either burst or fell off from the trees after injection. As these mangoes could not be taken into account while considering the results, the wastage from each sample is deducted from the respective population of samples actually injected. The numbers thus arrived at are given in the fifth column under the heading " net number ". These figures have been utilised for calculating the results.

TABLE I

Effect of injecting healthy Dasehri and Safeda mangoes
with ' old ' extract from naturally occurring necrotic mangoes
(extract ampouled in June 1938)

Date of injection		Variety of mango	No. of mangoes injected	Wastage after injection	Net No.	No. of mangoes showing early stage of necrosis	No. of mangoes showing advanced necrosis	Total No. of mangoes showing necrosis
27–4–1939	..	Safeda	12	2	10	5	5	10
27–4–1939	..	Dasehri	12	Nil	12	Nil	3	3
4–5–1939	..	Safeda	20	4	16	2	6	8
14–5–1939	..	Safeda	15	1	14	4	2	6
14–5–1939	..	Dasehri	10	1	9	Nil	3	3
16–5–1939	..	Safeda	25	2	23	7	3	10
TOTAL	..		94	10	84	18	22	40

It will be seen from Table I that out of ninety-four mangoes (Dasehri and Safeda) injected with old extract, ten were waste and out of the remaining eighty-four mangoes forty showed necrosis.

TABLE II

Effect of injecting healthy Dasehri and Safeda mangoes
with fresh extract from naturally occurring necrotic mangoes
(extract ampouled in May 1939)

Date of injection		Variety of mango	No. of mangoes injected	Wastage after injection	Net No.	No. of mangoes showing early stage of necrosis	No. of mangoes showing advanced necrosis	Total No. of mangoes showing necrosis
8–5–1939	..	Safeda	25	2	23	Nil	12	12
8–5–1939	..	Dasehri	25	6	19	Nil	3	3
14–5–1939	..	Dasehri	8	Nil	8	Nil	2	2
17–5–1939	..	Dasehri	10	Nil	10	Nil	1	1
24–5–1939	..	Safeda	25	2	23	1	6	7
27–5–1939	..	Safeda	20	1	19	5	1	6
TOTAL	..		113	11	102	6	25	31

It will be seen from Table II that out of a total of 113 mangoes (Dasehri and Safeda) injected with fresh extract from diseased tissue, there was wastage of eleven mangoes leaving 102 fruits, out of which thirty-one showed necrosis.

When the effect of injection on Dasehri and Safeda varieties is separately considered, it is seen that out of forty-three Dasehri mangoes injected with fresh extract, six were waste and only six showed necrosis. On the other hand, out of seventy Safedas similarly injected five were waste and twenty-five showed necrosis (Table II). Similar results were obtained with the old extract (Table I). Out of twenty-two Dasehri mangoes injected one was waste and six showed necrosis whereas out of seventy-two Safedas as many as thirty-four were affected.

That the state of maturity of the fruit has some effect on the incidence of disease is well illustrated by the results obtained from injections made at different periods. It is seen from Table II that out of twenty-three Safeda mangoes (leaving out wastage of two fruits) injected with fresh extract on 8–5–39, twelve were definitely necrotic. Safedas injected sixteen days later produced infection in lesser number showing only seven cases of necrosis out of twenty-three (two fruits being waste) and those injected after nineteen days produced necrosis in six out of a total number of nineteen (one fruit being waste). As against these are given the results of the control experiments in Tables III, IV and V.

TABLE III

Effect of injecting healthy Dasehri and Safeda mangoes with extracts from healthy mango fruits

Date of injection	Variety of mango	No. of mangoes injected	Wastage after injection	Net No.	No. of mangoes showing early stage of necrosis	No. of mangoes showing advanced necrosis	Total No. of mangoes showing necrosis
22–5–1939 ..	Safeda	20	Nil	20	3	1	4
27–5–1939 ..	Dasehri	10	Nil	10	Nil	Nil	Nil
27–5–1939 ..	Safeda	20	Nil	20	Nil	Nil	Nil
1–6–1939 ..	Safeda	20	Nil	20	Nil	Nil	Nil
3–6–1939 ..	Safeda	20	Nil	20	Nil	Nil	Nil
TOTAL ..		90	Nil	90	3	1	4

It will be seen from Table III that out of a total of ninety Dasehri and Safeda mangoes injected with the extract from healthy tissue, three showed early stage and one showed a slightly advanced stage of necrosis.

TABLE IV

Effect of injecting saline water into healthy mango fruits

Date of injection	Variety of mango	No. of mangoes injected	Wastage after. injection	Net No.	No. of mangoes showing early stage of necrosis	No. of mangoes showing advanced necrosis	Total No. of mangoes showing necrosis
8–5–1939 ..	Safeda	20	1	19	2	Nil	2
8–5–1939 ..	Dasehri	10	Nil	'10	Nil	Nil	Nil
14–5–1939 ..	Safeda	20	Nil	20	Nil	Nil	Nil
TOTAL ..		50	1	49	2	Nil	2

Table IV shows that out of fifty mangoes injected with normal saline, only one was waste and two showed small irregular water-soaked areas, which is the early stage of necrosis. These however did not advance any further.

TABLE V

Effect of blank injections on healthy mango fruits

Date of injection	Variety of mango	No. of mangoes injected	Wastage after injection	Net No.	No. of mangoes showing early stage of necrosis	No. of mangoes showing advanced necrosis	Total No. of mangoes showing necrosis
8–5–1939 ..	Safeda	10	1	9	Nil	Nil	Nil
18–5–1939 ..	Safeda	20	Nil	20	Nil	Nil	Nil
22–5–1939 ..	Safeda	25	2	23	Nil	Nil	Nil
22–5–1939 ..	Dasehri	5	Nil	5	Nil	Nil	Nil
TOTAL ..		60	3	57	Nil	Nil	Nil

Table V shows that out of sixty mangoes, which were given blank injections four were waste and none showed any necrosis.

Symptoms of Necrosis caused by Injection.—The foregoing results show that the extract from naturally occurring necrotic mangoes when injected

into healthy mango fruits (Dasehri and Safeda) produces necrosis in a large percentage of cases. The symptoms of necrosis produced are given below.

The first external symptom caused by the injection of extract from diseased tissue into healthy mango fruits was the appearance at the distal end, of small scattered, water-soaked areas of indistinct outline which gradually coalesced (Plate IV, Figs. 1–3). At a later stage brown colouration appeared at one or more parts of the water-soaked areas. These brown spots grew larger and coalesced forming what has been arbitrarily called the early stage of necrosis (Plate IV, Figs. 2–6). Complete coalescence of the brown spots occurred in more advanced stage of the disease. The necrotic area thus formed gradually changed into dark-brown and frequently assumed an ætiolated area round it. The disease advanced still further in some cases and the epicarp and mesocarp of the fruits were completely destroyed exposing the dark-brown, necrotic internal tissue but in no case was the emergence of seed observed (Plate IV, Figs. 12 and 13).

Only a very limited number of Safeda mangoes passed through all the stages mentioned above. The earlier stages, namely, the appearance of water-soaked areas and their coalescence were shown by a relatively small number of injected mangoes while the majority showed the advanced stage. The final stage of necrosis (the destruction of the infected tissue) was very rarely observed. A few injected Safedas directly produced the advanced stage of browning without apparently passing through the earlier stages. In the case of Dasehri too, only the advanced stage of necrosis was seen (Plate IV, Figs. 14–16).

Discussion

The experimental data presented in the foregoing pages indicate that healthy mangoes when injected with extract obtained from the mango fruits affected with necrosis developed a disease comparable to the necrosis of the mango fruit described in the previous paper of this series (Das Gupta and Verma, 1939).

A comparison of these results with those of the control experiments shows that there is a significant difference between the results obtained in the two cases. Taking all the control experiments together (Tables III, IV and V) it is found that altogether six mangoes developed necrosis out of a total of 197 mangoes* (three being rejected as wastage). Of these six mangoes, four developed necrosis when injected with extract from healthy tissue and

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* In this discussion the number given as injected denotes the net number obtained after deducting the wastage after injection.

two mangoes when injected with normal saline. Whereas out of a total population of 186 mangoes injected with 'old' and 'fresh' extracts seventy-one developed necrosis.

Of the two varieties of mangoes utilised for the experiment Safeda showed definitely more susceptibility to the extract than the Dasehri as shown by the respective number developing the disease (Tables VI and VII). Thus, for example, out of a total population of 128 Safedas injected fifty-nine showed the necrosis (46·1%) and out of a total population of fifty-eight Dasehri injected only twelve developed necrosis (20·7%). The result is different from that found with the naturally occurring necrosis where the Dasehri variety is more susceptible than the Safeda variety.

TABLE VI

Showing the results of Safeda mangoes variously injected

Substance used for injection	Net No. of injected Safedas	No. developing necrosis	% developing necrosis
Extract from diseased tissue ..	128	59	46·1
Extract from healthy tissue ..	80	4	5
Normal saline	39	2	5
Blank injection	52	0	0

TABLE VII

Showing the results of Dasehri mangoes variously injected

Substance used for injection	Net No. of injected Dasehri	Number developing necrosis	% developing necrosis
Extract of diseased tissue ..	58	12	20·7
Extract of healthy tissue ..	10	0	0
Normal saline ..	10	0	0
Blank injection ..	5	0	0

When Safeda and Dasehri are considered separately and compared with their respective controls, it is found that in both the cases significantly larger number of mangoes injected with the extract produces necrosis than the

mangoes used as control. The proportion in the case of Safeda is 46·1% to 5% and in the case of Dasehri 20·7% to 0·0%. The absolute freedom of the Dasehri mangoes from the disease in control experiments is to be particularly observed.

The inequality in the population of two varieties of mangoes (Safeda and Dasehri) inoculated is due to the lesser number of Dasehri trees available for investigation and also because Dasehri trees had borne relatively far fewer mangoes.

In control experiments with healthy extract and normal saline only a negligible number (5%) have shown the disease. The results are un-expected and cannot be accounted for except by assuming some contamination with the extract from the disease tissue or by assuming some slight metabolic disturbance due to the fluids concerned. Further investigation on this point will be made in this mango season.

The degree of susceptibility seems to be intimately related to the stage of development of the fruits as will be seen from Tables VIII and IX for Safeda and Dasehri respectively arranged from the data given in Tables I and II.

TABLE VIII

Showing the effect of injecting Safeda mangoes at given intervals of time

Extract injected	Date of injection	Net No. of injected Safeda	No. showing necrosis	% showing necrosis
Old ..	27-4-1939	10	10	100
Old ..	4-5-1939	16	8	50
Fresh ..	8-5-1939	23	12	50
Old ..	14-5-1939	14	6	43
Old ..	15-5-1939	23	10	44
Fresh ..	24-5-1939	23	7	30·4
Fresh ..	27-5-1939	19	6	31·6

TABLE IX

*Showing the effect of injected Dasehri mangoes
at given intervals of time*

Extract injected	Date of inoculation	Net Number of injected Dasehri	Number showing necrosis	% showing necrosis
Old ..	27–4–1939	12	3	25
Fresh ..	8–5–1939	19	3	16
Old ..	14–5–1939	9	3	33
Fresh ..	14–5–1939	8	2	25
Fresh ..	17–5–1939	10	1	10

The results are more regular for the Safeda than for the Dasehri.

The relative potency of the two extracts the ' old ' and the ' fresh ' can also be ascertained from Tables VIII and IX.   Samples injected on the same date by the two extracts, for example Dasehri on 14–5–1939 do not show any significant difference.   Neither do the Safeda mangoes inoculated at an interval of four days, on 4–5–1939 with old extract and on 8-5–1939 with fresh extract, show any difference.

The highest number of mangoes showing necrosis in any one sample was found to be ten out of ten (100%) in Safedas injected with old virus. Nevertheless in most of the cases the greater majority of mangoes remained unaffected by the injected extract.   Most probably this is due to the advanced state of maturity of the fruit which has been shown to have considerable effect on susceptibility.

A large majority of the affected mangoes showed the more advanced symptoms, only a few developed the final stage.   A relatively smaller number of affected mangoes failed to develop symptoms beyond those of the early stage.   Out of a total of seventy-one mangoes forty-seven showed the advanced stage and twenty-four showed the early stage.   All these twenty-four mangoes were Safedas.   It is important to note that in many cases the advanced stage in Safeda developed apparently without passing through the early stage.   In Dasehri variety the early stage was never observed.

A comparison of the necrosis that is experimentally produced by the injection of the extracts with necrosis described in the first paper of this

series shows that the early symptoms of naturally occurring necrosis are different from the necrosis produced experimentally. Aetiolation, the earliest symptom, which invariably appears in the former does not appear under experimental conditions. Similarly the water-soaked areas which develop under experimental conditions do not develop in necrosis occurring in nature. But the appearance of scattered grey spots which gradually coalesce to form the necrotic area and the ætiolated zone round it are similar in both. The symptoms of the more advanced stages of the disease are also similar.

The extent to which the fruits were affected by the disease was very much less in experimentally-produced necrosis than in naturally occurring ones. Safeda showed only a limited disintegration of the affected tissues of the distal end, while, in Dasehri the disintegration of the tissue was slightly greater. But in no instance was the emergence of seed observed.

Summary

The paper deals with the effect of injecting healthy Dasehri and Safeda mango fruits with the extracts from naturally occurring necrotic mangoes.

The experiment was carried out with mangoes undetached from trees. The injections were made at the distal end of the fruit.

Two passages one parallel to the long axis of the fruit, the other at right angle and intersecting each other were made by a needle of ·08 mm. external diameter. The extract was injected at one end by means of a syringe.

Two different extracts were used one prepared in 1938 and stored at a temperature ranging between 10–15° C. (old extract) and another freshly prepared in 1939.

Out of a total population of 186 mangoes (Dasehri and Safeda) inoculated with extract from necrotic tissue seventy-one or approximately 38% developed necrosis.

Safeda mangoes proved to be more susceptible to the injected extract producing necrosis in 46·1% mangoes than Dasehri where 20·7% proved to be necrotic.

Control experiments using extracts from healthy mangoes, normal saline and blank injections produced necrosis in four out of eighty (5%), two out of thirty-nine (5%) and nil out of fifty-two (0%) mangoes respectively.

Susceptibility is shown to be related to the state of maturity of the fruit.

The 'old' extract of 1938 showed effect similar to that of freshly prepared extract of 1939.

The symptoms of necrosis produced experimentally by injection are described and compared with those found in naturally occurring necrosis.

Acknowledgments

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DESCRIPTION OF PLATE FIGURES

FIGS. 1–13 *Safeda Variety*

In the figures the points of injection (maximum three) are shown by small dark-brown spots. The absence of one or all such points of injection in some figures is either due to the obliteration caused by the advanced state of necrosis or to the particular view chosen for showing the symptoms of necrosis to the best advantage.

FIG. 1. Appearance of water-soaked spots.

FIG. 2. Brown colour appearing within watery spots.

FIG. 3. Coalescence of water-soaked spots.

FIG. 4. Numerous brown-coloured spots at the tip on water-soaked areas.

FIG. 5. Coalescence of dark-brown spots.

FIG. 6. A more advanced stage of coalescence of dark-brown spots and advancement of necrosis.

FIG. 7. Coalesced spots turning into black with aetiolated skin around it.

FIG. 8. Formation of continuous necrotic area due to coalescence of dark-brown spots.

FIGS. 9–11. More advanced necrosis with aetiolated area around it.

FIGS. 12–13. Stages in the emergence of flesh owing to the collapse of healthy tissue.

Figs. 14–16 *Dasehri Variety*

Fig. 14. Showing dark-brown necrotic tissue at the distal end and aetiolation around it.

FIG. 15. The dark-brown necrotic tissue has invaded more of the tip region.

FIG. 16. A very advanced stage of necrosis where the dark-brown flesh of the fruit is exposed.

Addendum

TABLE X

Effect of injecting healthy Safeda mangoes with extract from naturally occurring necrotic mangoes

Date of injection	Number of mangoes injected	Wastage after injecting	Net number	No re-action	No. of mangoes showing early stage of necrosis	Number of mangoes showing advanced necrosis	Total number of mangoes showing necrosis
19–4–1940	50	22	28	10	10	8	18
2–5–1940	50	11	39	16	8	15	23
12–5–1940	50	13	37	21	15	1	16
15–5–1940	30	5	25	17	8	..	8

Effect of blank injections on healthy mango fruits

Date of injection	Number of mangoes injected	Wastage after injecting	Net number	No re-action	No. of mangoes showing early stage of necrosis	Number of mangoes showing advanced necrosis	Total number of mangoes showing necrosis
2–5–1940	40	16	24	21	3	..	3
12–5–1940	50	20	30	27	3	..	3
15–5–1940	30	9	21	21	..	..	..

The results obtained from the injection experiments carried out in 1940 are given in Table X. They fully confirm the results of the previous years.

S. N. Das Gupta and
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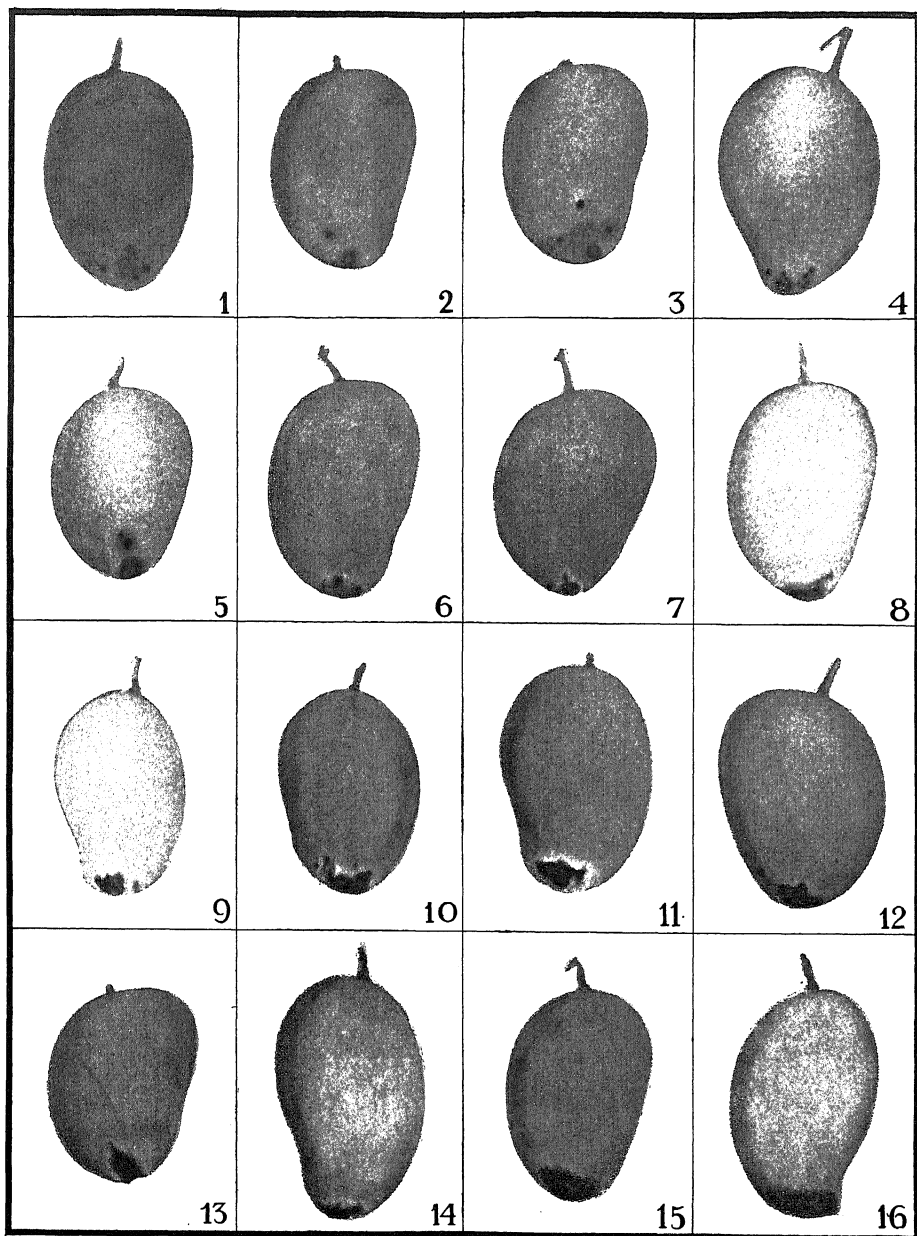
THE ESTIMATION OF RECESSIVE GENE FREQUENCIES BY INBREEDING

By J. B. S. Haldane

WE shall only concern ourselves with genes which are too rare for homozygotes to occur in appreciable numbers in a sample of the wild population and therefore also in the F_1 cultures derived from it. On the other hand the genes under consideration must be common enough to occur in the heterozygous condition in several members of the population. In *Drosophila* many of the genes occur only once in a sample of hundreds of flies, and we are concerned more with the total frequency of recessives than with the frequency of any individual recessive. Further we shall confine ourselves to genes which have normal viability and complete penetrance, so that when they segregate they segregate in one quarter of the members of a culture.

The progeny of a wild pair will be called a family consisting of one F_1 culture or sibship, and k F_2 cultures numbering $s_1, s_2, s_3, \cdots s_i, \cdots s_k$. There may also be F_3 cultures. A culture is said to be positive as regards a given recessive gene if that gene occurs in homozygous form in at least one member of it, and similarly with a family. A family or culture which would show the recessive phenotype if it were large enough will be called potentially positive.

What is the probability that a potentially positive family (necessarily derived from one homozygous and one heterozygous parent) will be negative? The probability that any of the k F_2 cultures is potentially positive is $\frac{1}{4}$. So if all the F_2 cultures numbered 40 or over, the desired probability would be very nearly $(\frac{3}{4})^k$. But of the potentially positive cultures $(\frac{3}{4})^s$ are negative. Hence the probability of a given F_2 culture being negative is $p(s) = \frac{3}{4} + \frac{1}{4}(\frac{3}{4})^s$. $p(s)$ and its logarithm are tabulated in Table I. The probability that a particular family, say the rth, though potentially positive, will be actually negative, is

$$P_r = \prod_{i=1}^{k} [\tfrac{3}{4} + \tfrac{1}{4}(\tfrac{3}{4})^{s_i}] = \prod_{i=1}^{k} p(s_i) \tag{1}$$

Let $Q_r = 1 - P_r$. We shall see later how equation (1) is to be modified if F_3 cultures are grown.

J. B. S. Haldane

TABLE I

s	$p(s)$	$\log p(s)$	s	$p(s)$	$\log p(s)$
1	·9375	·971971	17	·751880	·876149
2	·890625	·949695	18	·751410	·875877
3	·855469	·932204	19	·751057	·875673
4	·829102	·918607	20	·750793	·875521
5	·809327	·908126	21	·750595	·875406
6	·794495	·900088	22	·750446	·875319
7	·783371	·893967	23	·750335	·875258
8	·775028	·889318	24	·750251	·875206
9	·768771	·885796	25	·750188	·875171
10	·764078	·883139	26	·750141	·875143
11	·760559	·881133	27	·750106	·875123
12	·757919	·879323	28	·750079	·875107
13	·755939	·878486	29	·750059	·875095
14	·754455	·877633	30	·750045	·875087
15	·753343	·876993	∞	·75	·875061
16	·752707	·876511			

Now consider the case where in a set of families, m are positive for a given gene, while n are negative with Q_r values Q_1, Q_2, $\cdots Q_r$, $\cdots Q_n$. We desire to estimate the frequency x of potentially positive families, which is twice the frequency of the recessive gene per gamete. The probability that a given culture will be positive is xQ_r, the probability that it will be negative is $1 - xQ_r$. Hence the logarithm of the likelihood of the observed set of results is

$$L = m \log x + \sum_{r=1}^{n} \log (1 - xQ_r) + \text{constant.}$$

This is maximal when

$$\frac{dL}{dx} = \frac{m}{x} - \sum_{r=1}^{n} \frac{Q_r}{1 - xQ_r} = 0,$$

whence

$$m = x \sum_{r=1}^{n} Q_r + x^2 \sum_{r=1}^{n} Q_r^2 + x^3 \sum_{r=1}^{n} Q_r^3 + \cdots,$$

or

$$x = \frac{m}{\Sigma Q_r + x \Sigma Q_r^2 + x^2 \Sigma Q_r^3 \cdots} \tag{2}$$

When x is small the infinite series converges fairly quickly, and equation (2) is most readily solved by iteration. Let x_1 be the positive root of

$$x^2 \Sigma \, Q_r^2 + x \Sigma \, Q_r = m,$$

and let

$$x_2 = \frac{m}{\Sigma \, Q_r + x_1 \, \Sigma \, Q_r^2 + x_1^2 \, \Sigma \, Q_r^3 + \cdots}$$

x_3, x_4, etc., may be similarly calculated, and the process is shortened when it is remembered that the convergents are alternately larger and smaller than the final value. If $\hat{x}$ be the solution of equation (2) we have for the amount of information concerning it:

$$\sigma^{-2} = I = - \frac{d^2 L}{d\hat{x}^2} = \frac{m}{\hat{x}^2} + \sum_{r=1}^{n} \frac{Q_r^2}{(1 - \hat{x} Q_r)^2} \qquad (3)$$

$$= \frac{1}{x} \sum_{r=1}^{n} \frac{Q_r}{(1 - x Q_r)^2}$$

In some families further information is available because some pairs of F_2 sibs have been inbred, and have produced positive or negative F_3 cultures. We must ask ourselves how this diminishes the value of P_r if the cultures are negative. Supposing that out of a negative F_2 culture of s members, l pairs have been bred together and produced negative cultures of t_1, t_2, $\cdots$ t_j, $\cdots$ t_l members, how will this diminish the factor $p(s)$ which the F_2 culture contributes to P_r? If the F_2 culture is potentially positive, $\frac{2}{3}$ of its members are heterozygous, and 4/9 of the F_3 cultures potentially positive, as compared with $\frac{1}{4}$ of the F_2 cultures. Of the potentially positive F_3 cultures containing t members $(\frac{3}{4})^t$ are negative. Now the probability that two F_2 members of a potentially segregating culture are both dominants is 9/16. The probability that they are both dominant and also yield a negative F_3 of t members is

$$\tfrac{9}{16} \left[\tfrac{5}{9} + \tfrac{4}{9} (\tfrac{3}{4})^t \right] = \tfrac{5}{16} + \tfrac{1}{4} (\tfrac{3}{4})^t = h(t).$$

This quantity and its logarithm are tabulated in Table II. It follows that for $p(s)$ we must substitute

$$p'(s) = \tfrac{3}{4} + \tfrac{1}{4} (\tfrac{3}{4})^{s-2l} \prod_{j=1}^{l} h(t_j)$$

Logarithms of $\frac{1}{4} (\frac{3}{4})^{s'}$ are tabulated in Table III.

As before $P_r = \prod_{r=1}^{k} p'(s)$. The later calculations are unaffected. It is worth noting that if s exceeds 30, $p(s)$ is so close to $\frac{3}{4}$ that there is no need to make a correction for F_3. Actually Philip obtained no recessives with normal segregation in F_2 when s exceeded 16.

J. B. S. Haldane

TABLE II

t	$h(t)$	$\log h(t)$	t	$h(t)$	$\log h(t)$
1	·500000	·6989700	17	·314380	·4974549
2	·453125	·6562180	18	·313910	·4968052
3	·417969	·6211441	19	·313557	·4963165
4	·391602	·5928449	20	·313095	·4959507
5	·371825	·5703386	21	·313095	·4956762
6	·356995	·5526622	22	·312946	·4954694
7	·345871	·5389142	23	·312835	·4953154
8	·337528	·5283098	24	·312751	·4951987
9	·331271	·5201833	25	·312688	·4951112
10	·326578	·5139809	26	·312641	·4950459
11	·323059	·5092819	27	·312606	·4949973
12	·320419	·5057183	28	·312579	·4949598
13	·318439	·5020163	29	·312559	·4949126
14	·316955	·5009977	30	·312545	·4949126
15	·315843	·4994712	∞	·3125	·4948500
16	·315007	·4983203			

As an example of the numerical working, consider the case of w (white eye) in *Dermestes vulpinus* (Philip's data). Here $m = 9$, $n = 23$,

$$\Sigma Q = 10 \cdot 0833, \quad \Sigma Q^2 = 5 \cdot 0485, \quad \Sigma Q^3 = 2 \cdot 9203, \quad \Sigma Q^4 = 1 \cdot 6294,$$
$$\Sigma Q^5 = \cdot 9943, \quad \Sigma Q^6 = \cdot 6235.$$

The ratios of successive terms in this series are $\cdot 500$, $\cdot 551$, $\cdot 586$, $\cdot 610$, $\cdot 627$, gradually rising to $\cdot 730$, the largest value of Q_r. We shall make only a very slight error if we take the subsequent terms as a geometric series with common ratio $\cdot 65$. Our equation is then

$$x = \frac{9}{10 \cdot 0833 + 5 \cdot 0485\,x + 2 \cdot 7802\,x^2 + 1 \cdot 6294\,x^3 + \cdot 9943\,x^4 + \dfrac{\cdot 6235\,x^5}{1 - \cdot 65x}}.$$

The positive root of $5x^2 + 10x + 9$ is $\cdot 673$. Putting $x_1 = \frac{2}{3}$, and substituting in the right-hand side we have $x_2 = \cdot 580$. Substituting $\cdot 60$ on the right-hand side we have $x_3 = \cdot 613$. Substituting $\cdot 61$ we have $x_3 = \cdot 608$.

TABLE III

s'	$\frac{1}{4}(\frac{3}{4})^{s'}$	$\log \frac{1}{4}(\frac{3}{4})^{s'}$	s'	$\frac{1}{4}(\frac{3}{4})^{s'}$	$\log \frac{1}{4}(\frac{3}{4})^{s'}$
1	·1875	$\overline{1}$·1480780	16	·00253	$\overline{3}$·4031205
2	·14063	$\overline{1}$·0221701	17	·00190	$\overline{3}$·2787536
3	·10548	$\overline{2}$·8981765	18	·00143	$\overline{3}$·1553360
4	·07910	$\overline{2}$·7732743	19	·00108	$\overline{3}$·0334238
5	·05933	$\overline{2}$·6483600	20	·00080	$\overline{4}$·9030900
6	·04450	$\overline{2}$·5234863	21	·00060	$\overline{4}$·7781513
7	·03338	$\overline{2}$·3984608	22	·00045	$\overline{4}$·6532125
8	·02503	$\overline{2}$·3736956	23	·00035	$\overline{4}$·5440680
9	·01878	$\overline{2}$·1486027	24	·00028	$\overline{4}$·4471580
10	·01408	$\overline{2}$·0232525	25	·00020	$\overline{4}$·3010300
11	·01055	$\overline{3}$·8992732	26	·00015	$\overline{4}$·1760913
12	·00793	$\overline{3}$·7781513	27	·00013	$\overline{4}$·1139434
13	·00600	$\overline{3}$·6512780	28	·00010	$\overline{4}$·0000000
14	·00448	$\overline{3}$·5250448	29	·00008	$\overline{5}$·9030900
15	·00335	$\overline{3}$·4031205	30	·00001	$\overline{5}$·0000000

Substituting ·609 we have $x_4 = $ ·6089. We may take this as our final value. This corresponds to the presence of w in 15·22% of gametes.

The standard error of x is given by

$$I = 1/x \,(\Sigma Q_r + 2\,x\Sigma Q_r^2 + 3\,x^2\Sigma Q_r^3 + \cdots)$$
$$= 36\cdot28$$
$$\therefore \quad \sigma = \cdot1660.$$

Hence the frequency per gamete of w is $15\cdot22 \pm 4\cdot15\%$. With such a large standard error $15 \pm 4\%$ is a sufficiently accurate expression.

The question arises how to design an experiment so as to obtain the maximum amount of information with the minimum of work. The unit process of work is the mating of a pair and breeding and classification of their offspring. First consider the case when all cultures are large. If a family consists of one F_1 culture and k F_2 cultures, $k + 1$ cultures have been made. $Q = 1 - (\frac{3}{4})^k$, and $I = Q/x\,(1 - xQ)^2$. The efficiency of the estimation of x may be measured by the amount of information per culture,

or $I/k + 1$. Successive values of this for $k = 1, 2, 3, 4$, are:

$$2/x (4 - x)^2, \; 111/3 \, x (16 - 7 \, x)^2, \; 592/x (64 - 37 \, x)^2 \text{ and}$$
$$8960/x (256 - 175 \, x)^2.$$

When x is small these approximate to:

$$\cdot 125/x + \cdot 0625, \; \cdot 1458/x + \cdot 1276, \; \cdot 1445/x + \cdot 1671, \; \cdot 1367/x + \cdot 1841.$$

Hence the greatest efficiency is obtained by growing 2 or 3 F_2 cultures per family. Let us now consider what happens when F_2 cultures are small. Suppose each consists of only 4 members, then $Q = 1 - \cdot 829102^k$. The first four values of $I / k + 1$ approximate to:

$$\cdot 08545/x + \cdot 02921, \; \cdot 10420/x + \cdot 065414, \; \cdot 10752/x + \cdot 09248,$$
$$\cdot 10549/x + \cdot 11125.$$

Thus the greatest efficiency is attained by growing 3 or 4 F_2 cultures. It is very rarely worth growing an F_3. The above calculations are based on two assumptions. First it is assumed that wild individuals can be caught without difficulty, and secondly that the genes segregate in the normal ratio of 1 homozygous recessive in 4. If either wild individuals are hard to get, or recessives appear with a frequency of much less than $\frac{1}{4}$, then it may be worthwhile breeding larger numbers of cultures. Again, the arrangement of work may leave time for breeding F_3, but no time for breeding more F_2. If so the parents of the F_3 should obviously be chosen from the smallest F_2 cultures.

A NOTE ON THE RELATIVE POSITIONS OF THE CORPUS CALLOSUM AND THE HIPPOCAMPAL FORMATION

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ABBIE (1939) has recently questioned the classical hypothesis of Elliot Smith on the relation of the corpus callosum to the hippocampus and the lamina terminalis in the callosal mammals. The fibres of the corpus callosum, according to Elliot Smith, reach the opposite hemisphere by invading the lamina terminalis in the region of the dorsal (hippocampal) commissure which is itself ventral to the hippocampal area on the medial surface of the cerebral hemisphere. They make use of the commissural bed and when these fibres become the huge corpus callosum, the commissural bed is expanded and stretched. The study of the human embryological material supported this hypothesis. Streeter (1912) found that as the corpus callosum expanded " the mass spreads open a space for itself, in which process, a portion of the pre-commissural body is appropriated, and the eventual lamina terminalis presents a large surface in the median section including the whole corpus callosum and septum pellucidum ". [Quoted from Kappers, Huber and Crosby (1936).] Elliot Smith's hypothesis explained certain peculiarities as the formation of fornix dorsalis, stria Lancisii, fibræ perforantes, etc., which are too well known to be repeated here. It really formed the fundamental basis for the forebrain morphology and was widely accepted.

Abbie's theory is a complete repudiation of this classical view. According to him the callosal fibres do not traverse through the hippocampal commissure. On the contrary they pass through the subicular area which is the junctional area between the neopallium and the hippocampus. It means that the corpus callosum lies dorsal to the hippocampal area. Consequently the supracallosal indusium is not hippocampal remnant, but it is a part of the subicular cortex. Another consequential deduction is that the septum pellucidum is not derived from the paraterminal body but it is formed by a drawing up of the anterior part of the lamina terminalis only and there is no such thing as an open cavum pellucidum. The cavum itself is the result of dehesion in the lamina terminalis as a result of stress on it by the growing corpus callosum.

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The writer was preparing a detailed discussion on this new hypothesis—for he has had the opportunity of studying the forebrains of the mammals which Abbie has used for his paper—when there appeared a paper by Goldby (1940), which has anticipated most of the writer's objections. So the present note is only confined to the observations on the hedge-hog's brain, which Goldby has not touched upon and which Abbie has used in his papers.
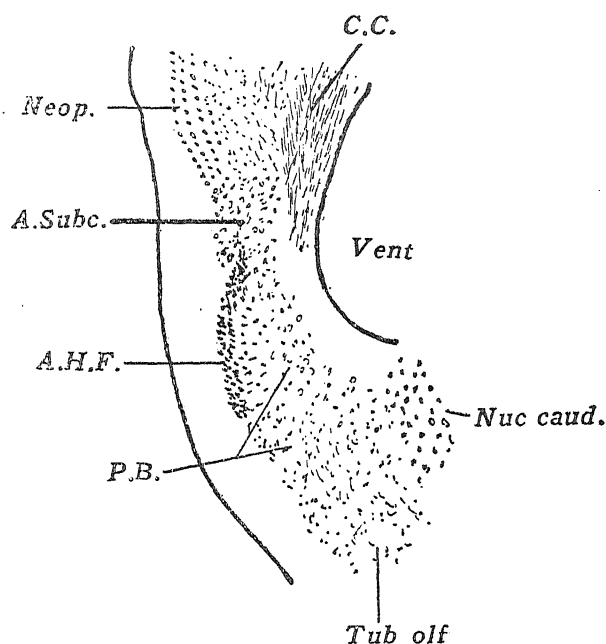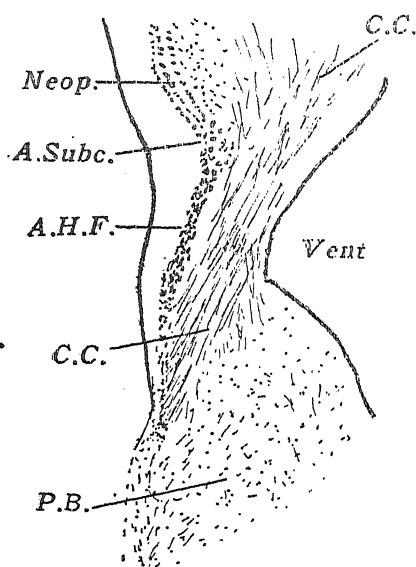
Fig. 1   Fig. 2

ABBREVIATIONS USED IN DIAGRAMS

A.H.F.	Anterior Hippocampal formation or area
C.C.	Corpus Callosum
P.B.	Paraterminal Body
A.Subc.	Area subiculum
Neop.	Neopallium
F.H.	Fissura Hippocampi
Nuc caud	Caudate nucleus
Tub. olf	Tuberculum olfactorium
Vent	Ventricle

Abbie seems to have made a fundamental error in wrongly identifying the hippocampal area as the subiculum. There seems to be some confusion in his labelling of the hippocampal and subicular areas in his two papers, published in the volumes 70 and 68 of the *Journal of Comparative Neurology*. (Compare the figures illustrating the sections of the hedgehog's brain in the

two papers.) In his 1939 paper on the corpus callosum, he has labelled the deeply staining compact layer of the hippocampal cortex as the subiculum, which is really a small junctional region of scattered cells lying between the dorsal edge of the hippocampal area and neocortex. From the study of the hedgehog's material at the writer's disposal, it is clear that a great part of the dorsal portion of the hippocampal cortex is lifted up by the anterior part of the corpus callosum and it is really the hippocampal area that is broken through by the developing fibres of the corpus callosum. The accompanying camera lucida drawings of this area in transverse section are self-explanatory and show that Abbie's theory is based on wrong interpretation of the medial cortical areas. So the present writer is in entire agreement with Goldby that Abbie has to produce more convincing evidence to substantiate his new hypothesis as to the relation of the corpus callosum to the hippocampus.

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EXPERIMENTS ON THE CONTROL OF SMUT OF SUGARCANE (*USTILAGO SCITAMINEA* SYD.)

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CONTENTS

I. *Introductory*

THE work on the systematic study of the smut of sugarcane (*Ustilago scitaminea* Syd.) was taken in hand in 1934. A paper on the life-history and modes of perpetuation of the disease has already been published (1938) elsewhere. On the basis of the work recorded in the previous paper, experiments on the control of this disease were carried out at Lyallpur and Risalewala. This paper gives an account of the work done during the last few years on measures of control which have been found effective against the disease and which can now be recommended to farmers.

II. *Control Measures*

Butler (1906), Ajrekar (1916), Lee and Medalla (1922) and Shepherd (1924) have made some casual observations on the control of this disease, but no systematic work seems to have hitherto been carried out.

The authors in their previous paper (1938) had shown that the smut of sugarcane perpetuates by the following methods:—

1. By planting setts taken from smutted shoots of canes.
2. By spores borne on the surface of buds of setts.
3. By the dormant mycelium inside the buds of setts in the standing canes.
4. By ratooning the smutted canes.

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The control of these sources of infection is dealt with below:—

1. *Avoiding planting of setts from smutted canes—*

As planting of setts from the smutted shoots of canes invariably leads to smut it is imperative that every care should be taken to avoid planting setts from diseased canes.

2. *Disinfection of setts before planting—*

Canes in the vicinity of smutted shoots may only apparently be healthy because such canes have often been found to carry living spores on their buds. Moreover if sufficient care is not taken at the time of harvest and smutted shoots are allowed to mix freely with the healthy canes, the spores may be carried to the buds and cause infection on planting. To guard against this it is necessary either to get seed canes from a smut-free locality or to disinfect the seed setts before planting. As no locality is likely to be quite free from smut, stress is laid on the disinfection of the setts before planting. In order to find out suitable disinfectants for this purpose, the following experiments were conducted:—

(*a*) *Effect of some selected disinfectants on the germination of smut spores.*—In order to determine the effect of copper sulphate, mercuric chloride and formalin solutions on the germination of smut spores various concentrations of these disinfectants were tried. Smut spores were shaken in the particular solution of the substance in a glass tube for a specified time. The suspension was then filtered through ordinary filter paper. The treated spores were thus left on the filter paper as residue. This residue was washed several times with distilled water for about 20 minutes. The spores were then picked up by means of a needle and sown in drops of distilled water. Observations on the germination of these spores were made for over seven days. The results obtained are presented in Table I.

From the results given in Table I the following conclusions can be drawn:—

1. In the case of copper sulphate the minimum strength of the solution which can kill smut spores after an immersion of 15 minutes is 0·2 per cent. In 1·0 per cent. solution, however, the spores are killed if the immersion lasts for 5 minutes only.

2. In the case of mercuric chloride, 0·05 per cent. and 0·1 per cent. are the minimum strengths of the solution which can destroy the germination power of the smut spores after immersions of 5 minutes and 3 minutes respectively.

3. In the case of formalin, solutions of 1·0, 2·0 and 3·0 per cent. can kill the spores after immersions of 30, 10 and 5 minutes respectively

TABLE I

Effect of disinfectants on the germination of smut spores

Disinfectant	% strength of solution	Period of immersion of spores in minutes	% germination of spores
Copper sulphate ..	2·0	5	0
	1·0	5	0
	0·5	15	0
	0·5	10	2·0
	0·2	15	0
	0·2	10	24·0
	0·1	15	30·0
Mercuric chloride ..	0·25	3	0
	0·1	3	0
	0·05	5	0
	0·05	3	30·0
	0·01	5	41·0
Formalin ..	1·0	30	0
	0·5	30	2·0
	2·0	10	0
	1·0	10	10·0
	3·0	5	0
	2·0	5	5·0
	0·5	Spores immersed for 5 minutes and covered for one hour under moist cloth.	0
	0·25	do.	48·0
	0·5	*	0
	0·25	*	2·0
Distilled water			98·3

* Spores immersed for 5 minutes and covered for one hour and 40 minutes under moist cloth.

and a concentration of 0·5 per cent. can destroy the germination power of spores if after an immersion of 5 minutes they are kept covered with moist cloth for about two hours.

(*b*) *Effect of disinfectants on the germination of sugarcane setts.*—Field experiments were arranged to ascertain the effect of three disinfectants—copper sulphate, mercuric chloride and formalin. Healthy setts of *katha* and Co. 285 cane with sound buds were selected and dipped in 0·05–1·0 per cent. solutions of mercuric chloride for 5 minutes. Setts thus treated were planted immediately.

Treatment with formalin was done by first dipping the setts for 5 minutes in 0·5–4·0 per cent. solutions and then covering the setts with moist gunny bags for two hours before planting.

In the case of copper sulphate the solutions were made in earthen pots and the immersion lasted for 15 minutes. The setts after treatment were sown in the field. Germination records were taken regularly and the final results are given in Table II.

From the results given in Table II it is clear that 0·05–1·0 per cent. solutions of mercuric chloride and 0·5–4·0 per cent. formalin solutions used in the experiments did not have any harmful effect on the germination of sugarcane setts. However, 0·5–2·0 per cent. solutions of copper sulphate depressed germination.

Disinfection of setts in 0·1 per cent. mercuric chloride solution for 5 minutes or in 1·0 per cent. formalin solution for the same period followed by two hours' covering under moist cloth in the case of the formalin treatment can, therefore, be safely recommended to the cultivators after making a due allowance for the errors which may result in their hands due to the faulty preparation of solutions or the inadequate duration of the treatment.

(*c*) *Treatment of setts artificially smeared with smut spores.*—Experiments were carried out in the field by artificially smearing setts with smut spores and then disinfecting them in 0·1 per cent. mercuric chloride solution and in 1·0 per cent. formalin solution, for 5 minutes followed by two hours' covering under moist gunny sacks in the latter case. The results obtained are given in Table III from which it is clear that both these treatments are effective in controlling surface borne infection and do not affect the germination of setts.

(*d*) *Effect of recommended treatments on yield.*—Experiments were conducted on a field scale to see the effect of recommended disinfectants on the yield of the sugarcane crop. For this purpose setts of variety Co. 285 were treated with 0·1 per cent. mercuric chloride solution for 5 minutes and with 1·0 per cent. formalin solution for 5 minutes followed by two hours' covering under moist gunny bags in the latter case. The treated and control setts were planted in randomised plots, each 1/80th of an acre in area, with four replications. The experiment was carried out in 1937–38 and 1938–39.

TABLE II

Effect of disinfectants on the germination of sugarcane setts

Variety	Disinfectant	Strength %	Time	No. of buds planted	No. of buds germinated	% germination	No. of buds planted	No. of buds germinated	% germination	Average germination %
				1935			1936			
Katha	Mercuric chloride	1·0	5 mts.	99	36	36·3	900	476	52·8	44·5
		0·5	5 ,,	98	45	45·9	900	475	52·7	49·3
		0·25	5 ,,	100	49	49·0	900	473	52·5	50·7
		0·1	5 ,,	99	53	53·5	900	528	58·6	56·0
		0·05	5 ,,	98	53	54·0	900	501	55·6	54·3
	Control ..			200	74	37·0	900	426	47·3	42·1
	Formalin ..	4·0	2hrs.	98	37	37·7	900	445	49·3	43·5
		3·0	2 ,,	99	46	46·4	900	462	51·3	43·8
		2·0	2 ,,	98	29	29·5	900	452	51·3	40·4
		1·0	2 ,,	99	39	39·3	900	510	56·6	47·9
		0·5	2 ,,	97	48	49·4	900	501	55·6	52·5
	Control ..			100	44	44·0	900	443	49·2	46·6
	Copper sulphate	2·0	15 mts.	60	9	14·3	..	..	..	14·3
		1·0	15 ,,	60	15	25·0	..	..	..	25·0
		0·5	15 ,,	60	26	43·3	..	..	..	43·3
	Control ..			60	31	51·6	..	..	..	51·6
				1937			1938			
Co. 285	Mercuric chloride	0·1	5 mts.	5046	2167	42·9	5504	3241	58·8	50·8
	Control ..			5068	2147	42·4	5500	3246	59·0	50·7
	Formalin ..	1·0	2 hrs.	5958	2212	43·7	5518	3231	58·5	51·1

TABLE III

Results of treatment of spore-smeared setts with disinfectants

Year	Treatment	No. of buds planted	No. of buds germinated	% germination	No. of smutted plants	% Smut
1936	(i) Buds of setts smeared with spores at planting time	300	123	41·0	17	13·8
	(ii) Buds of setts smeared with spores and dipped in water for 5 minutes before planting	320	127	39·6	31	24·4
	(iii) Buds of setts smeared with spores and dipped in 0·1 per cent. mercuric chloride solution for 5 minutes	430	153	38·2	0	0
	(iv) Healthy setts (Control)	820	326	39·7	0	0
1937	(i) Buds of setts smeared with spores at planting time	160	66	41·2	13	19·7
	(ii) Buds of setts smeared with spores and dipped in water for 5 minutes before planting	105	47	44·7	17	36·1
	(iii) Buds of setts smeared with spores and dipped in 0·1 per cent. mercuric chloride solution for 5 minutes	220	97	44·0	0	0
	(iv) Buds of setts smeared with spores and dipped in 1·0 per cent. formalin solution for 5 minutes and covered for 2 hours under moist cloth	120	54	45·0	0	0
	(v) Healthy setts (Control)	100	44	44·0	0	0

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The results of yield obtained from these plots are given in Table IV.

TABLE IV

Effect of disinfectants on the yield of sugarcane

Treatment	No. of buds planted	No. of buds germinated	% germination	Weight of stripped cane		Weight of juice	
				Mds.	Srs.	Mds.	Srs.
1937–38							
0·1% mercuric chloride	1248	509	40·7	9	38	6	5
Control	1260	495	39·2	10	0	6	20
1·0% formalin	1282	547	42·6	10	29	6	36
0·1% mercuric chloride	1260	529	41·9	10	9	6	28
1·0% formalin	1258	541	43·0	10	39	6	39
Control	1276	550	43·1	11	28	6	31
1·0% formalin	1242	534	42·9	10	5	6	11
0·1% mercuric chloride	1270	558	43·9	9	31	6	9
Control	1266	570	45·0	10	27	6	34
0·1% mercuric chloride	1268	571	45·0	10	35	6	37
1·0% formalin	1276	590	46·2	11	20	6	29
Control	1266	532	42·0	8	34	5	22
1938–39							
Control	1320	785	59·4	7	14	4	21
1·0% formalin	1395	828	59·4	7	35	4	31
0·1% mercuric chloride	1408	836	59·3	7	22	4	35
1·0% formalin	1395	808	57·8	6	23	4	2
Control	1364	842	61·7	7	2	4	15
0·1% mercuric chloride	1355	814	60·0	8	34	5	8
Control	1386	830	59·8	8	20	5	0
0·1% mercuric chloride	1355	788	58·1	6	15	3	37
1·0% formalin	1364	798	58·5	8	2	5	0
0·1% mercuric chloride	1386	803	57·9	7	21	4	27
Control	1430	789	55·1	7	14	4	12
1·0% formalin	1364	797	58·4	6	38	4	3

The results of yield of stripped cane and juice were examined separately and the analyses of variance are given below:—

Due to	Degrees of freedom	Sums of squares	Mean square

1937–38

Analysis of variance (*stripped cane*)

Due to	Degrees of freedom	Sums of squares	Mean square
Blocks ..	3	1853·0	617·6
Treatments ..	2	1442·7	721·3
Error ..	6	7766·0	1294·3
Total ..	11	11061·7	

Analysis of variance (*juice*)

Due to	Degrees of freedom	Sums of squares	Mean square
Blocks ..	3	504·9	168·3
Treatments ..	2	312·0	156·0
Error ..	6	2385·4	398·2
Total ..	11	3202·3	

1938–39

Analysis of variance (*stripped cane*)

Due to	Degrees of freedom	Sums of squares	Mean square
Blocks ..	3	380	126·6
Treatments ..	2	182	91·0
Error ..	6	8942	1490·3
Total ..	11	9504	

Analysis of variance (*juice*)

Due to	Degrees of freedom	Sums of squares	Mean square
Blocks ..	3	376	125·3
Treatments ..	2	122	61·0
Error ..	6	2633	438·8
Total ..	11	3131	

From Fisher's Z table it may be seen that if the results are to be significant, the mean square due to treatment should be at least 5·143 times the mean square due to error. Hence the results for both the years are not significant.

It is therefore clear that the treatment of setts with 0·1 per cent. mercuric chloride solution or 1·0 per cent. formalin solution did not affect the yield of cane or of juice.

3. *Roguing out smutted canes from the field.—*

Smutted shoots present in a cane crop are the cause of spread of the disease and are, at the same time, a potent agent in carrying over the disease from year to year. Spores from the smutted shoots are blown about by wind and falling on the buds of other healthy canes germinate and infect them. About 5–20 per cent. of the infected buds sprout and produce smutted shoots during the same growing season. In this way the disease goes on spreading. The buds of standing canes which are infected by the mycelium in this way, but do not give rise to smutted shoots during the same growing season, produce, on planting, smutted shoots in the following season. Further the spores of smut, even if they do not infect the buds but just remain sticking to the surface, can cause infection when such buds are planted in the field. Also the setts from smutted shoots, on planting, invariably give rise to smutted canes.

It is, therefore, evident that roguing out of smutted canes as soon as they appear is extremely important with a view to prevent the spread of the disease as well as to reduce the amount of carry over. During roguing precautions should be taken so as to prevent the transfer of spores to other canes. In practice it has been found convenient to take a gunny bag along into the field. The smutted shoots are first put inside this sack and then cut. After that the affected canes are pulled out.

The smutted shoots thus collected are either burnt or buried deep in the ground.

Another method of disposal of diseased canes is to feed them to cattle as the viability of the spores has been found to be destroyed in the alimentary canal of the cattle, as is shown by the following experiments:

Smutted canes were brought from the field every day, chopped and, after mixing bout 50 seers of these with 10 seers of *bhusa,* were fed to two male buffaloes. The dung was carefully collected as soon as it was passed out. The experiment lasted for a week.

During the course of the experiment samples of dung were daily brought to the laboratory and on examination found to contain numerous spores.

Then four different concentrations (N/1, N/2, N/4, N/8 where N/1 = 2 grams of dung dissolved in 100 c.c. of distilled water) of this dung were made in distilled water and drops from these placed for germination trials. In no case did any germination occur although innumerable spores were present in the drops as seen under the microscope.

In order, further, to ascertain whether the spores were actually dead or whether the dung solutions were unfavourable for their germination the following trials were made:—

(i) Drops of solution containing dung obtained after feeding smutted canes were placed for germination. The solution was made in distilled water by adding one gram of dung to 100 c.c. of distilled water.

(ii) Drops of solution containing dung free from smut spores were placed for germination after adding fresh smut spores artificially. Here, too, the dung solution was of the same concentration as in (i), *i.e.*, one gram in 100 c.c. of distilled water.

No germination occurred in case (i) while there was free germination in case (ii). Hence it is clear that spores after passing through alimentary canal of the cattle lose viability.

4. *Discouraging the practice of ratooning the crop*—

The practice of ratooning is definitely undesirable from the point of view of control of smut as this practice always provides favourable conditions for the appearance and perpetuation of the disease. Where ratooning of diseased crop is practised for a number of years the disease must make a permanent footing and the crop would suffer to such an extent that remunerative yield could not be expected, from such a crop. The practice of ratooning should therefore be stopped in the case of diseased varieties of canes.

All these methods of control described in the preceding pages have been followed at the Sugarcane Research Station, Risalewala, Lyallpur, and the disease has been effectively controlled.

The methods of control which are recommended to the farmers are as follows:—

1. Setts from smutted canes should not be used for planting.

2. Seed setts should be disinfected in 0·1 per cent. mercuric chloride solution for 5 minutes or in 1·0 per cent. formalin solution for 5 minutes followed by two hours' covering under moist cloth in the case of the formalin treatment.

3. All smutted plants should be rogued out as soon as they appear.

4. Ratooning of diseased crops should be discouraged.

III. Summary

Measures to control the disease have been investigated.   The following have been found effective and are recommended to the farmers:—

(i) Setts from smutted canes should not be used for planting.

(ii) Seed setts should be disinfected in $0 \cdot 1$ per cent. mercuric chloride solution for 5 minutes or in $1 \cdot 0$ per cent. formalin solution for 5 minutes followed by two hours' covering under moist cloth in the case of the formalin treatment.   These treatments do not adversely affect either the germination of sugarcane setts or the yield of cane and juice.

(iii) All smutted plants should be rogued out as soon as they appear.

(iv) Ratooning of diseased crop should be discouraged.

Acknowledgment

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SOME PECULIARITIES IN CONJUGATION IN A NEW HIMALAYAN SPECIES OF *ZYGNEMA*

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(*Allahabad*)

AN interesting species of *Zygnema* was collected by the author from a fresh-water stream near Kapkot, district Almora, on the way to Pindari glacier on 14th September 1939. This alga shows a number of peculiarities in conjugation which it is desired to record in this paper.

The vegetative cells are on the average 21 μ broad and 3–6 times as long. Usually each cell contains two stellate chloroplasts, but in some cases cells containing four chloroplasts were also found.

Conjugation

Lateral conjugation is the predominant mode of conjugation in this species of *Zygnema*, though scalariform conjugation is also seen in some filaments. In some cases neighbouring cells may be found conjugating in a ladder-like and lateral fashion (Fig. 6). Between these two set modes of conjugation, we find a number of intermixed stages showing a conflict between two different sexual tendencies of this alga. In one case it was seen that though a zygospore had formed by the fusion of protoplasm from two opposite cells in the conjugation canal, still the chloroplasts and protoplasm from a neighbouring cell had invaded a portion of one of the cells in an abortive attempt to conjugate laterally (Fig. 5). An opposite case was also noticed where two neighbouring cells had conjugated laterally and produced an immature zygospore, and a contiguous cell of a neighbouring filament gave out a broad conjugation canal which could be seen attached to it.

In one instance it was seen that the terminal cell of a filament had fused with the middle part of another resulting in the formation of a zygospore surrounded by three arms of the gametangia (Fig. 4). This resembles the three-horned zygospores described by the present author in various species of *Zygnemopsis*.

Lateral Conjugation.—However it is in its peculiar mode of lateral conjugation that this alga differs from other species of *Zygnema* as well as other members of the order Zygnemales. In most of the laterally conjugating species of *Zygnema* and *Spirogyra* tent-like conjugation processes arise

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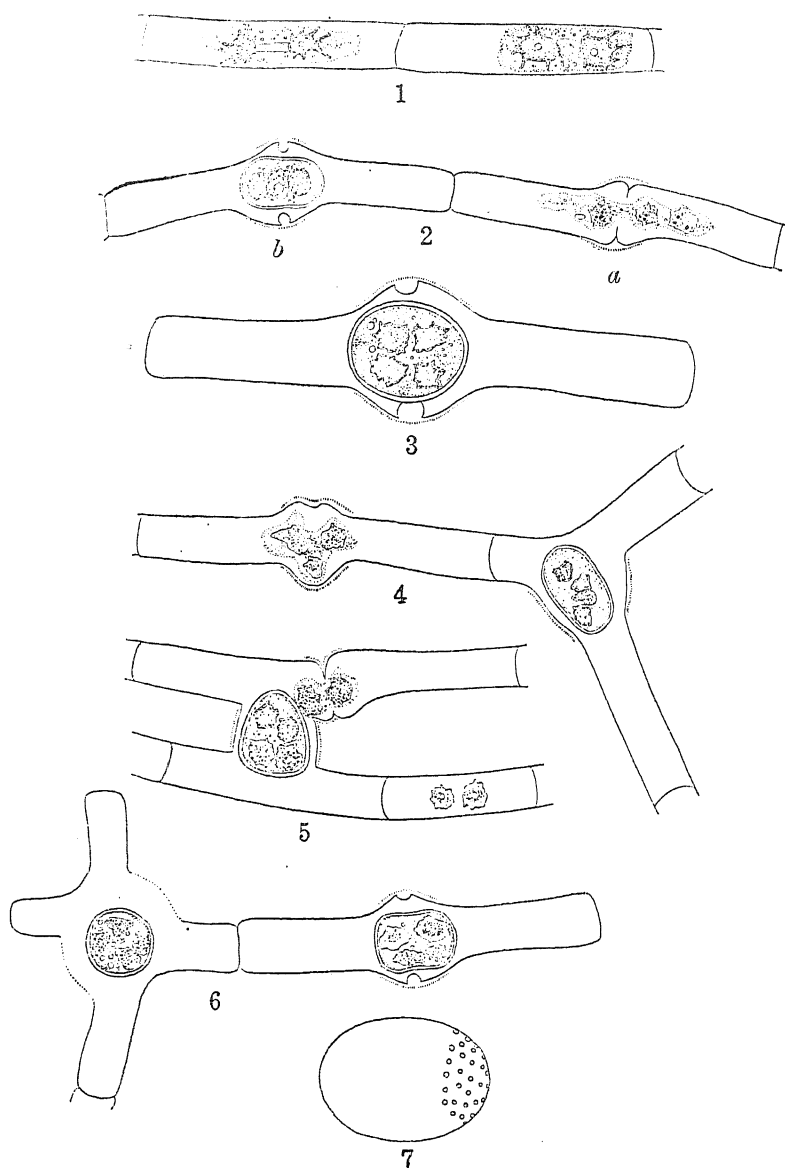
TEXT-FIGS. 1–7.   *Zygnema himalayensis* sp. nov.

1. Vegetative cells.   2. Early stages in lateral conjugation.   3. A later stage in lateral conjugation.   4. and 5. Abnormalities in conjugation.   6. Lateral and scalariform conjugation. in neighbouring cells.   7. A ripe spore.

All figs. are × 320 except 3 and 7 which are × 410.

from adjacent ends of neighbouring cells, as a result of a gradual protrusion of the longitudinal wall on either side of the septum. In the region of the protrusion the septum breaks down, and the male protoplast passes over to the female cell, where it fuses with the female protoplast. From among Indian forms such a mode of lateral conjugation is seen in *Zygnema mucigena* Randhawa.[4] This is an isogamous mode of lateral conjugation with the zygospore lodged in the female gametangium and is the commonest type prevalent.

Taking the various isogamous modes of conjugation in species of *Zygnema*, the most highly advanced type is seen in *Zygnema Heydrichii* Schmidle,[1] in which the conjugation canal area is cut off by a partition wall from the remaining parts of the gametangia, and the zygospore lies in a dome-like space. Comparatively more primitive is the mode of lateral conjugation seen in *Zygnema Czurdae* Randhawa[3] in which also the zygospore lies in the middle and no partition walls are formed. In this case however tent-like outgrowths are formed, and geniculation of cells takes place.

A third and the most primitive type of isogamous lateral conjugation is seen in the present Himalayan form. Unlike all the forms described above, no tent-like projections arise in the present form. In this alga lateral elongation of longitudinal walls takes place on all sides, rather than on one side. As a result the septum ruptures and the protoplasts and chloroplasts from both the mating cells move towards the middle (Fig. 2). This elongation of the side walls is evident from the fact that the cell-walls in the area of fusion bulge out considerably though the chloroplasts are still in a linear row showing the direction in which they have moved (Fig. *a*). Later on the chloroplasts become grouped in a quartette, and the spore-wall is secreted by the protoplasm (Fig. *b*).

The possibilities of such a direct mode of lateral isogamous conjugation were discussed by the present author in his description of *Zygnema terrestris* Randhawa.[4] However unlike the present form no actual fusions in a straight line were observed in that species. The mode of lateral conjugation observed in the present Himalayan form is the simplest and the most primitive described so far.

Mature zygospores are globose or sub-globose, are bluish-green in colour, 36 to 40 μ broad, and 45 to 72 μ long. The spore-wall is scrobiculate with pits 1 to 1$\frac{1}{2}$ μ in diameter and 3–4 μ apart (Fig. 7).

This alga differs from all the previously described species of *Zygnema* in its peculiar mode of lateral conjugation and position of zygospores,

and hence it is desirable to describe it as a new species which is called *Z. himalayensis.*

Zygnema himalayensis sp. nov.

Cellulis vegetativis 20–22 μ latis, 60–120 μ longis, conjugation laterali vel interdum scalari, cum fusione gametarum in medio ; zygosporis in tubo conjugationis, globosis vel sub globosis ; 36–40 μ latis, 45–72 μ longis, mesosporio coeruleus-viridio scrobiculato ; scorbiculis 1–1$\frac{1}{2}$ μ diametro, intervallis 3–4 μ.

Habit.—Fresh-water stream, Loharkhet, 5750 feet above sea level, Almora, 15th September 1939.

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A GENETICAL ANALYSIS OF THREE SMALL POPULATIONS OF *DERMESTES VULPINUS* F. (COLEOPTERA)

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THE kinds of evolution which are possible within a group of species are determined by its inherent variability, defined by the typical rates of all mutational changes. The result of these changes, the raw material of evolution, of which we still know very little, once it is released into a species, becomes subject to the laws which govern its ecological habits. The fate of newly arisen allelomorphs or chromosomal configurations depends on such conditions as the average size of the population into which they may spread, on the opportunities for migration into new surroundings, or for elimination in various circumstances, which may or may not take into account the relative fitness of its carrier (Dobzhansky, 1937). The future of a neutral gene differs in communities of different size, different degrees of migration, etc. If these variables are known, statistical treatment can give information on the trend which evolution must take in the particular species, as has been demonstrated in a certain number of theoretically possible populations by Haldane, Fisher and Wright. Actually very few accurate data on the genetical constitution of free-living populations are available. Populations of various species of *Drosophila* have been systematically inbred by a number of workers. Though nothing but an occasional visibly abnormal fly occurs, the majority of the flies caught were carriers of recessives in heterozygous form, which were recovered in the F_2 generation from matings outside the laboratory. The method employed in obtaining the flies was in most cases to trap the animals over comparatively large areas, perhaps with the exception of Timofeeff-Ressovsky's method. He caught his flies from a garbage can.

The population of a given species of insect in the comparatively large area of a field or an orchard is not continuous but falls into smaller communities, perhaps small feeding groups where the larvæ from one batch of eggs develop together. It must depend on the motility of the animal under investigation whether these sub-groups are stable or not. Insects of the type of *Drosophila* migrate probably both by active as well as by passive locomotion (Gordon, 1939). Therefore animals trapped under the conditions

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generally employed are only able to give a picture of a population which
has already undergone a secondary mixing process, the nature of which
we do not know, since the original one had not, and quite possibly could
not have been studied. The present investigation is concerned with the
beetle *Dermestes vulpinus* F., one of the common storehouse pests. It is
proposed to give an analysis of three more or less isolated breeding com-
munities, probably derived from a very limited number of animals and not
much disturbed by secondary migration.

Material

Fowler, in his work on the British Coleoptera, describes the imago of
*Dermestes vulpinus* as follows:

" Oblong, unicolorous, moderately shiny, elytra clothed with sparing
grey pubescence; head thickly and rather coarsely punctured, densely pubes-
cent; antennæ red or pitchy red; thorax rather long, considerably narrowed
in front, thickly and rather strongly punctured, clothed with whitish-grey
pubescence at sides, central portion almost bare; scutellum thickly covered
with orange-yellow hairs; elytra black thickly punctured, with apical sutural
angles mucronate; legs covered with brownish hairs; underside clothed with
long and thick white pubescence with a row of black spots at each side
near margins, last segment black with two white longitudinal patches; male
with a bunch of brownish bristles on fourth segment of abdomen. Length
6–9 mm." (Fig. 1).
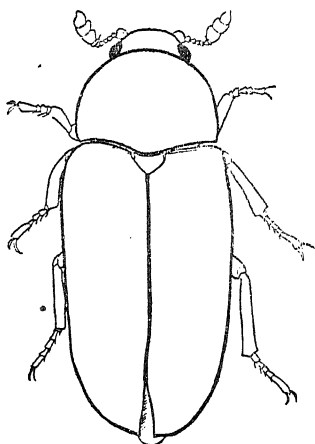
TEXT-FIG. 1. Normal Female

To this description may be added another secondary sexual character
which I have not found recorded anywhere in the literature accessible to me.

The first and the second pair of legs carry a brush of golden coloured hair on the first tarsal segment. All males show it without exception (Fig. 2).

(a)   Sex-comb   (b)
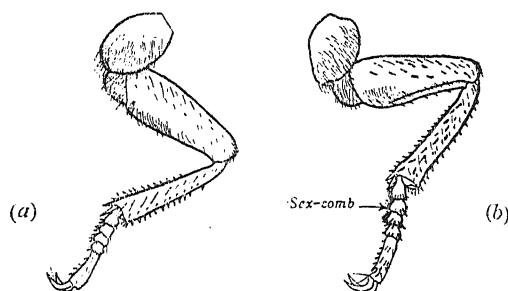
TEXT-FIG. 2. (a) Foreleg of Female.   (b) Foreleg of Male.

The eggs are cylindrical with rounded poles, slightly bent, about 2 mm. long.

A very thorough description of the larvæ is given by Kreyenberg (1928). Fowler's notes may suffice here. " The body is elongated, narrowed towards the tip and terminates in two corneous cerci and a conical appendage. The surface is very hairy, the colour brown or reddish-brown."

The pupæ are naked, ivory when freshly emerged, except for light-reddish-brown eyes and chitinous spines on the back. During the pupal stage the darkening and final differentiation of the imago can be observed (Fig. 3).
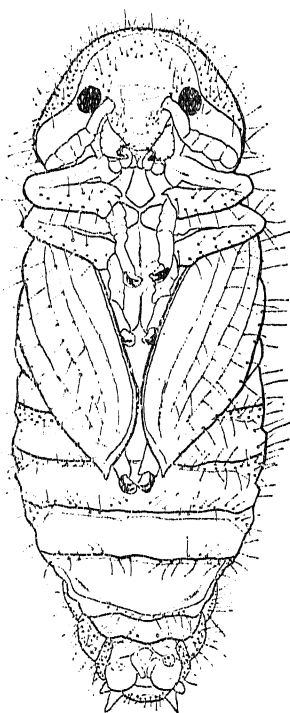
TEXT-FIG. 3. Normal Female Pupa

Biology and culture methods

The animals are kept in half-pint milk bottles, filled to a depth of one inch with fishmeal previously fumigated with CS_2. To provide sufficient moisture a vial containing a plug of moist cotton-wool is added. As the medium is apt to deteriorate, special care has to be taken to avoid direct contact between the fishmeal and the moist plug. But if the culture becomes too dry the development is severely retarded. The larvæ acquire canni-balistic habits, attacking specially the defenceless soft and juicy pupæ. A wad of dry cotton-wool gives the larvæ ample opportunity to burrow and to pupate in relative safety.

Bottles, vials, and cotton-wool are sterilised for half an hour in live steam in order to prevent the growth of mould. The bottles are closed with cardboard milk-tops.

Neither the larvæ nor the imago is able to climb clean glass, so that it is sufficient to leave a gap between the top and the cotton-wool to avoid escapes.

The cultures are kept in a constant temperature room at $25° \pm 1°$ centigrade and a humidity of about 90%.

Single pair matings were usually set up. It is convenient to separate the sexes in the pupal stage to ensure virginity. Under these conditions, eggs were laid 10–14 days after emergence. The young larvæ, white with red hairs, hatch after 3 or 4 days. These become coloured within a few hours. They first develop the black pigment only, which becomes denser with every following instar. After the third moult the yellowish red pigment of the dorsal stripe appears. On hatching every segment has two rows of bristles; with each successive instar an additional row is formed, up to the fifth moult, after which the number of bristles remains constant. The number of larval instars is not completely fixed, but depends to a certain degree on temperature, humidity and food. In my cultures the number of moults was usually seven in the female and six in the male. The time taken for the different instars varies from one stage to the next, the last stage being the longest.

During the first half of this last period the animal feeds actively and can only be discriminated from the preceding stages by its size. Later on it loses its activity, becomes strongly thigmotactic, abandons feeding and lies immobile in a little hollow till the emergence of the pupa. These pre-pupæ are easily distinguished from the other larvæ by their lack of bristles and the bent position which they always resume even after momentary disturbance.

At the end of this period the pupa emerges. The act of emergence is brought about by the expansion of the wings. In the pre-pupa, shortly before it has reached maturity, the elytra and wings can be seen as two small flaps of tissue growing out of the second and third thoracic segments. When blood is pumped into them, they stretch ventrally, wedging themselves between the last larval skin and the legs, thus exerting a pressure on the already thinned skin and bursting it in the back. This process of stretching the wings is only completed about an hour after emergence, after which the final position becomes fixed. Pupæ which do not emerge properly have unexpanded wings. The whole pupal period lasts 10–14 days. The gradual differentiation and colouring of the legs, mouthparts, etc., can be observed easily.

When the imago finally emerges from the pupa, it turns the wings from the ventral position to the dorsal one. At first it is very light yellow and orange. Full pigmentation is not achieved before another two days, during which the light colour changes gradually through deep red to black.

10–14 days after emergence the adult is sexually mature. The eggs are deposited in batches up to 24 corresponding to the number of ovarioles. But this number is often depleted by cannibalism of the female. The first batch is usually sterile, the majority of the eggs being deformed. It seems that there are individual differences between females determining the size and form of the eggs.

No diapause was observed under the conditions employed.

The method of keeping the cultures was as follows:—About four weeks after mating the larvæ were collected and set up in sub-cultures, only leaving larvæ of the same size together. The batches usually consist of 7–16 larvæ. The parents, if alive, are transferred into fresh bottles. If a culture is left without change the yield of adults becomes rather small, usually 10–12. Though a large number of larvæ may appear, they are destroyed by each following age group on reaching the pupal stage. Only the very last survive.

Description of the material

It was planned to analyse the genetical constitution of three small distinct populations of *Dermestes vulpinus*. The animals investigated came from isolated piles of sheepskins collected once in two successive summers (1937 and 1938).

Population 1937 was a sample from a heavy infection consisting of well over a thousand individuals, of which only a fraction were taken. The shipment came from the Argentine.

Population 1938 I was found inside a bale where animals had eaten a cavity. The infection was detected by a few animals on the outside of the pile. Most of the population was taken. The shipment came from Kenya.

Population 1938 II was collected from the outside of a pile. The infection had only started. The animals caught probably represent the resident beetle population of the store.

Such infections probably constitute fairly isolated breeding communities, very little disturbed by migration.

It is obvious that any attempt to estimate the genetical constitution of a group must be subject to severe methodological criticism. The capacity of the observer to detect differences is by no means constant, partly because in course of time the object becomes more familiar and types, which were missed in the beginning, are noticed later on, and partly because of personal variation in observing. The account of this piece of work can only be judged with these objections in mind.

Because of limitation in time imagines only were thoroughly investigated. A search for variations in eggs and larvæ requires a different technique.

The samples collected from the different populations consisted of:

TABLE I

		Imagines		Pupæ	Larvæ	Animals examined
		Dead	Alive			
Population 1937	..	..	80	201	60	341
Population 1938 I	..	97	87	14	96	237
Population 1938 II	..	..	14	..	24	38

Of the animals collected, mostly pupæ and larvæ were used for breeding purposes, as nearly all the adults were sterile. Though superficially intact, it is thought that they have suffered some damage during the transport.

Imagines hatched in the laboratory were set up in pairs. From the progeny of every original pair 8–10 pairs were again mated to produce the F_2 generation.

If both parents were heterozygous for the same allelomorphs, segregation was expected in the F_1 generation. In case only one of the parents was heterozygous for a fully penetrant and fully recessive factor one quarter of

the cultures was expected to show the mutant in the ratio of 1: 3. As a result of sampling a certain percentage of the mutants are missed. A correction factor has to be introduced to account for the loss of information in each family owing to the number and size of F_2 cultures reared (Haldane, 1940).

In this communication only F_1, F_2 and occasionally F_3 data are given. The fuller genetical analysis will follow in another paper.

Results

The population collected shows polymorphism for a number of characteristics, which on subsequent investigation proved to be inherited. No records of this were found in papers dealing with the systematics of the Dermestids with the exception of " light antennæ " by Fowler (1889).

Three of these characters were inherited in a simple mendelian manner.
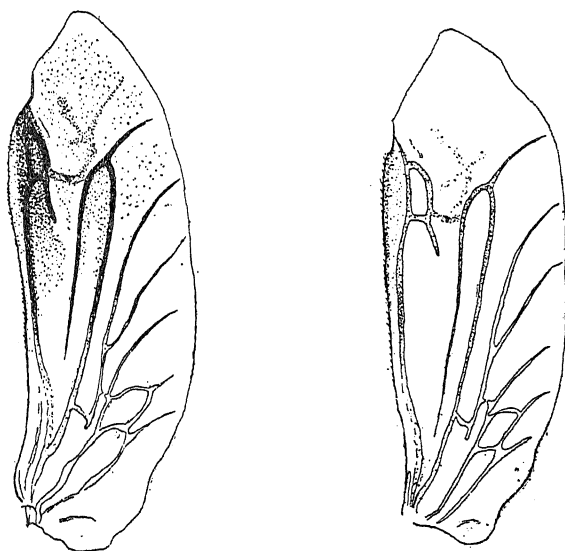
Text-Fig. 4. Dark (L) and Light (C) Wing from Text of *Genetics*, 38

(a) *Dark wing* (L)–*Light wing* (l) (Fig. 4).—Animals of the phenotype L exhibit a darkly pigmented costa, subcosta, radius, sector and, to a more varying degree, a pigmented cubitus and internomedia. Some diffuse pigment is present along the subcosta, in the radial cell, in the field bordered by the sector, and between the two branches of the cubitus, sometimes reaching the analis as well. The amount of pigment in and between the veins decreases with rising temperature, affecting first the analis, internomedia and cubitus and the diffuse pigment between them.

Animals of the phenotype *l* show slighter pigmentation in the costa, subcosta, radius and sector. Pigment in the marginal sectors of the cubitus is only developed at low temperatures. The diffuse pigment is reduced as well and confined to the subcosta, the radial cell and the field inside the sector. Here again the amount of pigment present decreases with increasing temperature.

At any given temperature the two types segregating in the same culture are distinct, though L-animals reared at 30° resemble *l*-animals reared at 18° (Phenocopy?).

L is dominant over *l*. The heterozygote L/*l* is indistinguishable from the homozygote L/L raised at 30° and 18°, but can be recognised if reared at 24°.

Beetles with dark and light wings were found in four different populations. They were originally discovered in the stocks reared at the Entomological Field Station of the Imperial College, London, and in the three populations described here.

It is very probable that populations 1937 and 1938 I came from the Argentine and Kenya respectively. In population 1938 I a good criterion for the origin of the population was found. The pigmentation of the wing is dependent on temperature. Among the dead animals a number showed no trace of colour. It must therefore be concluded that they had developed at a much higher temperature than is likely to be found in the storehouses of the Port of London. The age of the population must have been about 6–8 months at the time of collection in June. The infection therefore either took place in Kenya or on board ship.

The only drawing of a wing of *Dermestes vulpinus*, which is given by Bernet-Kempers (1923) records the light type, which was preponderant in each group so far investigated.

In *Dermestes lardarius* only dark-winged animals were observed.

Tables II*a* and II*b* give the F_1 and F_2 segregation from animals caught in the free-living populations. Though from the genetical analysis it is quite clear that the phenotype L differs from the phenotype *l* by a single pair of allelomorphs, the figures given in Tables II*a* and II*b* deviate considerably from the expected 3 L : 1 *l* respectively 1 L : 1 *l* ratios.

In population 1937 the ratios differ uniformly from the expected 1 : 1 ratio in crosses between L/*l* with *l*/*l*, but agree well with the 3 : 1 ratio from crosses between L/*l* with L/*l* individuals. The deviation from the 1 : 1 ratio is uniform throughout the cultures. This may partly be explained by the

TABLE IIa

F_1 and F_2 segregations of $L-l$

Cross: $L/l \times L/l$

Population	Generation	L	l	χ^2	Heterogeneity χ^2 between cultures	n	p	χ^2 total populations	Heterogeneity χ^2 between generations	n	p	Heterogeneity χ^2 between populations	n	p
1937	F_1	..	..	..	..	..		.39						
	F_2	288	89	·39	15·06	20	·8							
1938 I	F_1	32	11	·01	..			10·05	1·33	1	·25			
	F_2	362	11·37	11·37	46·04	27	>·01							
1938 II	F_1	..	..	..	..	14	·05	3·68						
	F_2	277	113	3·68	26·37									
Total		959	377	4·13	86·78	63	·05					7·99	2	·02

TABLE IIb

F_1 and F_2 segregations of $L-l$

Cross: $L/l \times l/l$

Population	Generation	L	l	χ^2	Heterogeneity χ^2 between cultures	n	p	χ^2 total populations	Heterogeneity χ^2 between generations	n	p	Heterogeneity χ^2 between populations	n	p
1937	F_1	287	215	10·33	34·28	25	·1	28·1	·06	1	·8			
	F_2	467	347	17·63	49·41	36	·13							
1938 I	F_1	120	116	·07	41·19	12	<·01	·26	·63	1	·4			
	F_2	148	164	·82	32·83	11	<·01							
1938 II	F_1	55	54	·01	21·16	6	<·01	·01	·06	1	·8			
	F_2	30	32	·06	·42	3	·9							
Total		1107	928	15·74	182·59	98	<·01					12·63	2	<·01

fact that culture conditions were not yet satisfactory and that there may have been a selection in favour of the L/l individuals.

In population 1938 I and II the totals show good agreement with the expectation but there is a marked heterogeneity between cultures. It was thought that possibly lethal factors linked to the locus of L may account for this heterogeneity. A number of egg abnormalities leading to the death of the embryo have been found in the stocks and may be tested at a future date. The two types of deviation account for the heterogeneity between the populations.

If a significant deviation is found the homozygous recessive class is usually favoured.

(b) *Black Body* (B)–*Brown Body* (b).—The black colour of the elytra and thorax in the genotype BB or B*b* becomes dark reddish-brown in the genotype *bb*.

The character is a difficult one to score because it resembles one stage of the colour changes the body undergoes during the first days after emergence. The pigment of the ventral side is black even in *bb* animals. This quality may be used to decide whether an imago is fully coloured or not. The amount of pigment decreases with rising temperature. The decrease is more marked in *bb* individuals. B is completely dominant over *b*.

Brown imagines (genotype *bb*) were found in Population 1937 and in Population 1938 II. The allelomorph *b* was completely absent in Population 1938 L.

In Tables III*a* and III*b* the F_1 and F_2 segregations of B–*b* from Populations 1937 and 1938 II are given in the cross B/*b* by B/*b* the heterogeneity between cultures is large though the totals are in good agreement with expectation. There is also a significant heterogeneity between populations.

Though the heterogeneity between cultures disappears in crosses B/*b* by *b*/*b* the heterogeneity between populations persists. The deviation from the 1:1 ratio in the totals is significant. In this case the homozygous recessive is deficient.

(c) *Dark antennæ* (A)–*Light antennæ* (a).—The three terminal joints of the antennæ are dark brown dorsally. Ventrally they may, as in A*a* or AA be dark-brown or may be light-brown as in *aa* individuals. From Fowler's description it is evident that he noticed both types.

TABLE IIIa

F_1 *and* F_2 *segregations of* B–b

Cross: $B|b \times B|b$

Population	Generation	B	b	χ^2	Heterogeneity χ^2 between cultures	n	p	χ^2 total populations	Heterogeneity χ^2 between generations	n	p	Heterogeneity χ^2 between populations	n	p
1937	F_1	120	46	1·19	23·15	9	<·01	3·8	2·30	1	>·1			
	F_2	199	88	4·91	19·77	15	·2							
1938 II	F_1	40	10	·66	·01	1	·9	1·76	·15	1	·7			
	F_2	469	140	1·31	62·89	18	<·01							
Total		828	284	·17	113·72	46	<·01					5·39	1	<·01

TABLE IIIb

F_1 *and* F_2 *segregations of* B–b

Cross: $B|b \times b|b$

Population	Generation	B	b	χ^2	Heterogeneity χ^2 between cultures	n	p	χ^2 total populations	Heterogeneity χ^2 between generations	n	p	Heterogeneity χ^2 between populations	n	p
1937	F_1	54	49	·24	6·27	12	·9	·73	·65	1	·4			
	F_2	168	149	1·14	11·35	14	·7							
1938 II	F_1	56	24	12·8	3·29	2	·2	14·01	1·29	1	·3			
	F_2	25	15	2·5	1·00	2	·4							
Total		303	237	8·07	30·52	33	·8					6·67	1	<·01

In Population 1937 imagines with " light antennæ " were only found when examining the second generation. Some of the F_2 cultures were homozygous for " a ". As " a " is completely recessive to " A ", " a " must have been present in the original sample. Both types were found in Population 1938 I and II.

Dermestes lardarius has uniformly light antennæ in three populations examined phenotypically.

In Tables IVa and IVb the segregations in Populations 1938 I and II only are given, because the families are not large enough to infer the type of mating from the progeny. There is marked inhomogeneity between single cultures, but fairly good agreement between the generations.

When dealing with material freshly isolated from its natural habitat, one must realise, that both genetical as well as ecological variables not directly under observation may disturb the segregations. Though it is not possible to isolate without considerably more extensive experimentation modifiers, lethals, chromosomal aberrations, etc., the homogeneity tests may point to such disturbances which are important for the understanding of the evolutionary processes.

Besides the three pairs of allelomorphs, described in the preceding paragraphs, some further variations were found in all three populations. Each was present in the original sample of at least one colony. They were therefore included in the list of characters for which *Dermestes vulpinus* is polymorphic.

(*d*) *Large radial cell (R)–Small radial cell (r)*.—The cell at the margin of the wing, formed by the recurrent radius and closed by a small cross vein is variable in size, due to the shifting of this cross vein. The character " r ", small radial cell was found in 5 of 341 animals, taken in 1937. It was not found in the other samples, but segregated on inbreeding.

From former experience it was known that the transmission of this factor is complicated. In order to determine preliminarily, how far environmental factors or a number of weak modifiers influence the appearance of the character, the occurrence of R–r in the three generations under observation in the three populations is given in Table V.

No significant increase of the phenotype " r " is observed from the P to the F_1 generation.

As the P and the F_1 generation resemble each other in the degree of inbreeding, the fact that the frequency of this condition is not materially

TABLE IVa

F_1 and F_2 segregations of A–a

Cross: A'a × A'a

Population	Generation	A	a	χ^2	Heterogeneity χ^2 between cultures	n	p	χ^2 total populations	Heterogeneity χ^2 between generations	n	p	Heterogeneity χ^2 between populations	n	p
1938 I	F_1	7	5	1·77	..	..	..	6·76	·88	1	·4			
	F_2	345	146	5·87	81·18	20	<·01							
1938 II	F_1	58	12	2·3	1·22	1	>·3	3·44	·13	1	·7			
	F_2	201	55	1·27	23·75	10	<·01							
Total		611	218	·77	116·59	33	<·01					10·44	1	<·01

TABLE IVb

F_1 and F_2 segregations of A–a

Cross: A/a × a/a

Population	Generation	A	a	χ^2	Heterogeneity χ^2 between cultures	n	p	χ^2 total populations	Heterogeneity χ^2 between generations	n	p	Heterogeneity χ^2 between populations	n	p
1938 I	F_1	111	149	5·55	29·18	11	<·01	2·79	2·85	1	·1			
	F_2	183	189	·09	22·36	14	·1							
1938 II	F_1	32	18	3·92	16·32	2	<·01	8·48	·88	1	·4			
	F_2	167	127	5·44	16·94	9	·05							
Total		493	483	·1	99·70	39	<·01					3·63	1	·05

increased, demonstrates that changed surroundings do not further the appearance of "*r*".

In Population 1937 a significant increase is observed from F_1 to F_2 generation, contrary to the findings in the other *p* populations.

In Table VI the F_2 segregations from matings R by R are given for the three populations. To compare these segregations, the deviation of the single matings from the expected 3 : 1 ratio was tested.

If environmental factors are responsible for the disturbed ratios little heterogeneity between cultures is expected, because culture conditions were kept as uniform as possible. As no progeny is grown in a single bottle, it is to be expected that variations in the bottles might cancel each other. If however genetical factors influence the segregation of the factor, a certain amount of heterogeneity has to be expected due to the segregation of the modifiers themselves.

The F_1 of Population 1937 if added up is in good agreement with the expected 3 : 1 ratio. The presence of some complicating element is indicated by the large value for heterogeneity.

In the other two populations the deviation from the 3 : 1 ratio is significant. There is no inhomogeneity.

(e) *Extra veins* (*ex*) (Fig. 5 *c–e*).—The normal wing of *Dermestes vulpinus* is shown in Figure 5 (*a*). This type of venation is found in many Coleoptera.
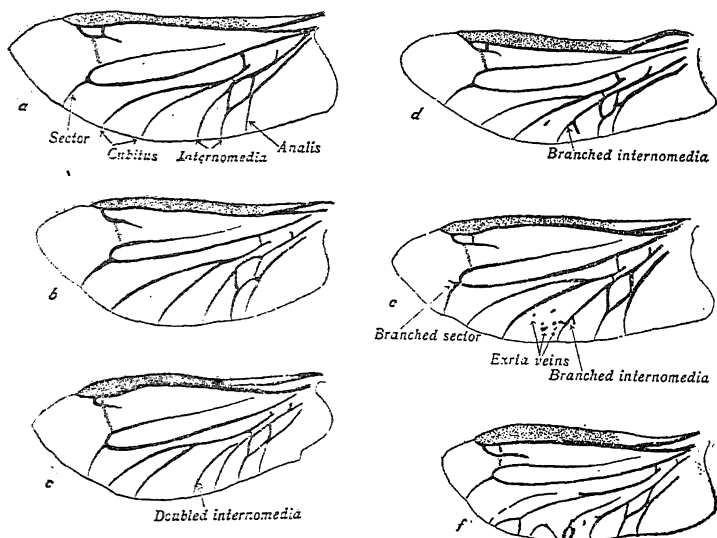
TEXT-FIG. 5

Table V
Occurrence of R–r in 3 generations

Population	P		F1		F2		Heterogeneity χ² between generations			Heterogeneity χ² between populations		
Phenotype	R	r	R	r	R	r	P/F1	F1/F2		P	F1	F2
1937	336	5	828	7	1521	77	·95	26·06	1937/38 I	4·18	1·97	79·12
1938 I	283	··	475	1	2601	17	·02	1·34	1937/38 II	·15	1·11	21·54
1938 II	38	··	173	3	880	11	·13	·25	1938 I/38 II	··	4·71	2·88

$\chi^2 = 3.84$ $P = ·05$ for $n = 2$

Table VI
F_2 *segregations of R–r*

Cross: R × r

Population	R ♀	R ♂	r ♀	r ♂	χ^2	n	p	Heterogeneity χ² between cultures	n	p
1937	109	130	39	38	·07	1	> ·8	40·09	17	< ·01
1938 I	68	73	2	15	17·09	1	< ·01	7·92	5	< ·2
1938 II	42	45	5	6	9·91	1	< ·01	2·09	3	< ·5

TABLE VII

Occurrence of "extra veins" (ex) in 3 generations

Generation Phenotype / Population	P		F₁		F₂		Heterogeneity χ^2 between generations			Heterogeneity χ^2 between populations		
	+ex	ex	+ex	ex	+ex	ex	P/F₁	F₁/F₂		P	F₁	F₂
1937	341	..	835	..	1598	36	..	8·87	1937/38 I	1·92	6·93	8·71
1938 I	280	3	471	5	2574	44	7·14	1·03	1937/38 II	·62	12·13	3·25
1938 II	37	1	176	3	885	6	·16	1·8	1938 I/38 II	7·15	·19	4·8

$\chi^2 = 3.84$ P $= \cdot05$ for $n = 2$.

TABLE VIII

F₂ segregations of +ex—ex

Cross	+ex × +ex									
Phenotype / Population	+ex		ex		χ^2	n	p	Heterogeneity χ^2 between cultures	n	p
	♀	♂	♀	♂						
1937	90	112	21	15	11·1	1	<·01	19·16	9	·02
1938 I	226	248	25	19	75·3	1	<·01	16·73	17	>·5
1938 II	73	47	5	1	27·5	1	<·01	1·26	3	>·7

The nearly related *Dermestes lardarius* has an almost identical pattern. In the aberration to be described here, some additional vein or fragments appear.

Costa, Subcosta and radius are not affected. The other veins: sector, cubitus, internomedia and analis may show a variety of changes especially in the distal sections.

Abnormalities classified:—

Sector:	A small spur.
Cubitus:	Branching of the distal sections of both arms in both directions, in extreme cases doubling of one branch. The duplicated segment may or may not join up with the main vein. The formation of the surplus vein may start proximally or distally. Sometimes a number of disconnected fragments lie between the veins.
Internomedia:	Same as cubitus.
Analis:	Same as cubitus, but no disconnected fragments.

The two wings are not necessarily affected simultaneously, and if both are affected not necessarily in the same manner. The character "extra veins" show varying penetrance and is not completely recessive.

Types comparable to "extra veins" are found abundantly in populations of *Dermestes lardarius*.

"Extra veins" occurs in the samples collected in 1938. It was found in 15% of the families of population 1937, in 36% of population 1938 I and in 12% of population 1938 II.

There was a significant increase from F_1 to F_2 population 1937 and one from P to F_1 in population 1938 I. In population 1938 II which, from its appearance, was the least inbred community, no significant increase was observed from one generation to the other.

In crosses normal by normal, which segregate for "extra veins", the heterogeneity is insignificant.

(f) *Irregular cubitus* (*cu*).—The branch 1 of the cubitus may not reach the margin of the wing, the vein may be interrupted in the lower third of its course or it may be doubled all along. The character does not appear

F

TABLE IX

Occurrence of " irregular cubitus " (cu) in 3 generations

Generation	P		F₁		F₂		Heterogeneity χ² between generations		Populations	Heterogeneity χ² between populations		
Phenotype	+cu	cu	+cu	cu	+cu	cu	P/F₁	F₁/F₂		P	F₁	F₂
1937	341	..	835	..	1627	7	..	3·59	1937/38 I	2·42	..	1·8
1938 I	281	2	476	..	2598	20	·63	3·66	1937/38 II	..	4·67	·06
1938 II	38	..	178	1	887	4	·21	·20	1938 I/1938 II	·27	2·66	·97

TABLE X

F₂ segregations of " irregular cubitus " (cu)

Cross	+cu × +cu				χ²	n	p	Heterogeneity χ² between cultures	n	p
Phenotype	+cu		cu							
Population	♀	♂	♀	♂						
1937	35	17	1	6	5·43	1	·02	·01	3	·9
1938 I	116	110	6	14	37·20	1	< ·01	2·59	10	·9
1938 II	32	36	3	1	9·8	1	< ·01	9·8	2	< ·05

TABLE XI

Occurrence of " irregular cross vein " (cv) in 3 generations

Generation Phenotype Population	P		F$_1$		F$_2$		Heterogeneity χ^2 between generations			Heterogeneity χ^2 between populations		
	+^{c}v	cv	+^{c}v	cv	+^{c}v	cv	P/F$_1$	F$_1$/F$_2$		P	F$_1$	F$_2$
1937	336	5	835	..	1618	16	10·04	6·35	1937/38 I	..	..	1·79
1938 I	283	..	476	..	2580	38	..	5·14	1937/38 II	..	..	6·9
1938 II	38	..	179	..	891	..	..	..	1938 I/1938 II	..	..	11·16

$\chi^2 = 3\cdot84$ P = ·05 $n = 2$

TABLE XII

F$_2$ segregations of +^{cv} − cv

Cross	+^{cv} × +^{cv}									
Phenotype Population	+^{cv}		cv		χ^2	n	p	Heterogeneity χ^2 between cultures	n	p
	♀	♂	♀	♂						
1937	76	55	9	7	15·62	1	<·01	5·64	7	·6
1938 I	247	299	24	14	104·3	1	<·01	18·18	16	·3
1938 II	..	..	..	..	..	..	..	..	..	..

symmetrically and is variable in penetrance. It seems distinct from the character "extra vein", though the relation to it is not quite clear.

No significant increase is found from one generation to the other. The frequencies within the populations do not differ appreciably.

In all three populations the deviation from the 3 : 1 ratio is significant but the heterogeneity is low.

(g) *Irregular cross vein* (*cv.*) (Fig. 5 *b*).—This complex includes changes in the position of the radial cross vein. This cross vein may either be absent, or partly absent or it may be doubled. The character is not always symmetrical, it may affect the wings differently, doubling the cross vein in one wing and deleting it in the other. It is variable in penetrance.

There is a significant increase of animals exhibiting the character from the F_1 to the F_2 generation.

It occurred in populations 1937 and 1938 I. The progeny of all matings normal by normal deviates uniformly from a 3 : 1 ratio. Besides the characters found in the original sample, a number of other factors were isolated in the F_2 generation.

Population 1937—

(*a*) *White eye* (*w*).—The normally dark eye is unpigmented and becomes transparent. The change can already be detected in the embryo shortly before hatching. A similar character has been described by Park in the beetle *Tribolium castaneum*.

TABLE XIII

F_2 *segregations of* " *white eye* " (*w*)

Cross	$+^W$ × $+^W$				χ^2
Phenotype	$+^W$		W		
	♀	♂	♀	♂	
12 Cultures	148	145	45	43	.73

" White eye " is completely recessive to dark eye. Viability is good.

(b) *Short elytræ* (*sh*) (Fig. 6).—The mucronate elytræ covering the whole of the abdomen are shortened by approximately the length of one segment
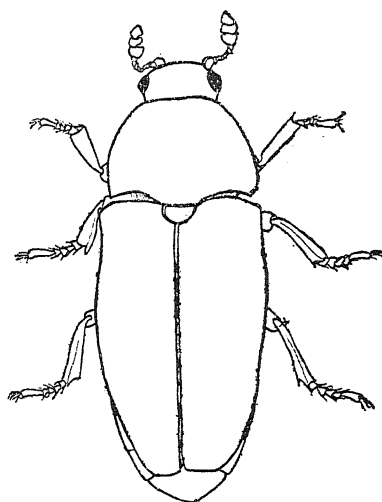
TEXT-FIG. 6. " Short Wing ", Male.

and become rounded at the tips. The wings are also shortened, though they may sometimes be too long to be tucked under completely. The mutant can be recognised in the pupa.
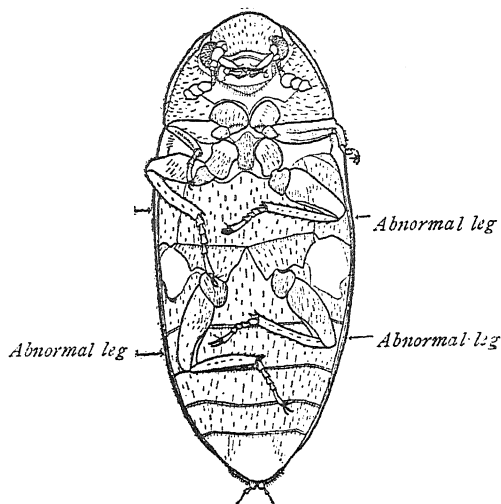
TABLE XIV

F₂ segregations of " short wing " (sh)

Cross	$+^{sh}$		$\times$	$+^{sh}$	χ^2
Phenotype	$+^{sh}$		sh		
	♀	♂	♀	♂	
4 Cultures	41	40	6	5	4·72

" Short elytræ " is completely recessive. Viability is bad.

(c) *Coxa vara* (*co*) (Fig. 7).—The different joints of the arthropod leg are capable of moving in one direction only.

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TEXT-FIG. 7. "Coxa vara" (co) Female

Body—Coxa joint	..	.. Upwards and downwards.
Coxa—Trochanter joint		.. Right to left, parallel to body.
Trochanter—Femur joint		.. Swivel motion.
Femur Tibia joint	..	.. From a wide to an acute angle in relation to the femur.

In the mutant "coxa vara", so called in analogy to the human knock knee, the movement of the Coxa Trochanter joint is disturbed in such a manner, that the leg is fixed in the extended position and thus cannot participate in the walking movements. Only the second and third pair are affected. The fore-legs are always normal. The abnormal legs are kept stretched parallel to the body. If only one of the hindlegs is affected, the movements are still fairly co-ordinated. But if several legs are paralysed the beetle becomes helpless on smooth surfaces and tumbles constantly to its back. It can only survive in a medium where it can burrow.

The manifestation is irregular. The legs are affected independently. In Fig. 7 an animal with three abnormal legs has been drawn. The two fore-legs 1 *a* and 1*b* are normal. The right leg of the second pair 2*a* shows the normal position in the etherised and thus relaxed animal. The other legs, 2 *b*, 3 *a* and 3 *b* are extended in the abnormal forced position. If the legs are extended flexed artificially they immediately return to the original extended position.

TABLE XV

F₂ segregation of " coxa vara " (co)

Cross	$+^{co}$	$\times$	$+^{co}$		χ^2
Phenotype	$+^{co}$		co		
	♀	♂	♀	♂	
4 Cultures	22	20	5	6	·63

The factor is fully recessive, but not fully penetrant. Viability is somewhat depressed.

(*d*) *Plexus* (*pl*) (Fig. 5*f*).—The " plexus " wings are shorter than the normal wings. The radial cell has become extremely small. All veins except the costa are affected. Cubitus, internomedia and analis branch in all possible directions. The wings do not fold properly and hang down at the side of the body. The elytræ do not close. The length of life of the " plexus " animals is reduced and the females are sterile. The character however is not fully penetrant and animals, which do not show it, or do not show it in a severe form are fertile.

" Plexus " was found in the F₃ of one family.

TABLE XVI

F₂ segregations of " plexus " (pl)

Cross ..	$+^{pl}$	$\times$	pl		χ^2
Phenotype .	$+^{pl}$		pl		
	♀	♂	♀	♂	
2 Cultures ..	24	26	4	7	1·72

(*e*) *Second sex-pit* (Fig. 8).—In *Dermestes vulpinus* the male may be distinguished from the female by a secondary sexual character, a bunch of brown bristles on the fourth abdominal segment. The *Dermestidæ* have been divided into two sub-groups, differing from each other by the presence or absence of a second sex-brush on the third abdominal segment.

One male appeared in one family in the third generation with a pit in the position typical for the second sex-brush in *Dermestes lardarius*. No bristles were present. The F₁ from this male were sterile and no stock was obtained. This aberration was found again in population 1938 I.

Population 1938 I—

(a) *Second sex-pit in males* (Fig. 8).—Males similar to the individual found in the F_3 of population 1937 were discovered during the inbreeding of population 1938 I.
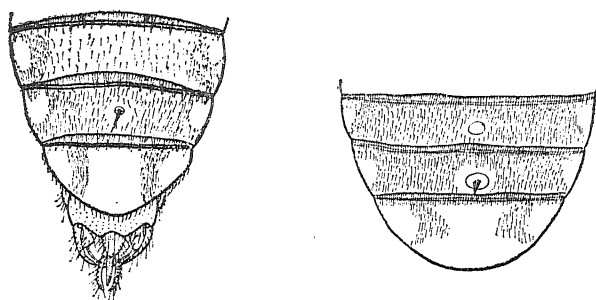
TEXT-FIG. 8. (a) Abdomen of Normal Male (b) Abdomen of ' Second Sex Pit ', Male

No males showing " second sex-pit " were seen in the sample collected. But in 5 of the F_1 cultures and in 15 altogether of the 22 families bred to a second generation, this abnormality occurred. The females are all normal.

The expression of the abnormality varies considerably. Occasionally there may be a clear pit with one or two bristles in it. Often a shallow depression is seen in the space where the pubescence is missing. Sometimes the pit only becomes visible when the hair is rubbed away. There are overlaps with the normal.

It is at the moment assumed that " second sex-pit " is inherited as an autosomal recessive with a sex limited action.

TABLE XVII

Occurrence of " Second sex-pit " (sp) in 3 generations

Generation	P		F_1		F_2		Heterogeneity χ^2 between generations	
							P/F_1	F_1/F_2
Phenotype	$+^{sp}$ ♂	sp ♂	$+^{sp}$ ♂	sp ♂	$+^{sp}$ ♂	sp ♂	P/F_1	F_1/F_2
	101	..	226	7	1262	109	1·43	7·26

There is a significant increase of " second sex-pit " males from the F_1 to the F_2 generation.

Under this assumption mating of normal females to normal males should give two kinds of segregations. If the female has been heterozygous for " second sex-pit " 75% of her sons should be normal and 25% abnormal. In case she had been homozygous a ratio of 50% normals to 50% abnormals is to be expected. The progeny of 39 crosses normal by normal seem mostly to have had a heterozygous mother. In only one cross the deviation from

the expected 3 : 1 ratio is significant. In 15 other crosses the segregation is compatible with both possible parental genotypes. The total from all crosses shows a fairly good agreement with a 3 : 1 ratio, and no appreciable heterogeneity. This suggests that not many females homozygous for " second sex-pit " had been present.

TABLE XVIII *a*
F$_2$ segregations of " second sex-pit " (sp)

Cross	$+^{sp}$ $\times$ $+^{sp}$		χ^2	Hetero-geneity χ^2	n	p
Phenotype	$+^{sp}$ $+^{sp}$	sp				
	♀ ♂	♂				
	458 385	105	3·3	48·2	38	·2

TABLE XVIII *b*
F$_2$ segregations of " second sex-pit " (sp)

Cross	$+^{sp}$ $\times$ sp		χ^2	Hetero-geneity χ^2	n	p
Phenotype	$+^{sp}$ $+^{sp}$	sp				
	♀ ♂	♂				
Segregation *a* ..	33 13	12	·04	3·95	3	·2
Segregation *b* ..	4 14	..	7·69	..	..	..
Segregation *c* ..	6 ..	10	10	..	..	..

From crosses: normal females by " second sex-pit " males, three types of segregation are expected: (*a*) Females homozygous for " second sex-pit " should have only abnormal sons. One family of 16 had 10 females and 6 abnormal males, a chance of one in 64 if the female had been heterozygous. (*b*) Heterozygous females should have 50% of abnormal sons. For such cultures were reared. Finally, (*c*) homozygous normal females should have no abnormal sons. One such progeny occurred in the F$_1$.

No exceptional sterility was noted in connection with " second sex-pit ".

(*b*) *White tip* (*wt*) (Fig. 9).—The ventral pattern of the fifth abdominal segment consists of two white bands enclosing two black marginal spots, leaving a black space in the middle. In the variation found in this population the space in the middle is filled in to a varying degree with a fine white pubescence.

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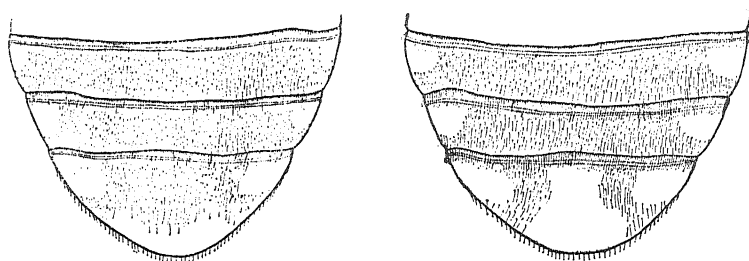
TEXT-FIG 9. (a) Abdomen of Normal Female   (b) Abdomen of " White tip " Female

The penetrance and expression of the factor is variable.

TABLE XIX

F_2 segregations for " white tip " (wt)

Cross ..	$+^{wt} \times +^{wt}$		χ^2	Heterogeneity		
Phenotypes ..				χ^2	p	n
Cultures ..						
I 3 (9)	16	2	1·85			
9 (12)	12	1	2·07			
26 (2)	5	5	3·35 ⎫	3·29	·05	1
(5)	21	3	·00 ⎭			
51 (2)	27	3	3·60 ⎫			
(6)	24	1	5·88 ⎬	·73	·7	2
(8)	10	2	·44 ⎭			
32 (2)	30	1	7·84			
37 (2)	17	9	1·28 ⎫	·08	·8	1
(7)	7	3	·13 ⎭			
14 (3)	15	13	6·85 ⎫			
(4)	9	12	11·57 ⎪			
(7)	13	24	22·35 ⎬	2·76	·6	4
(8)	12	4	·00 ⎪			
(9)	15	10	3·00 ⎭			
			70·21			
16 Cultures	245	102	3·09	67·12	< ·01	14
Without family I 14	185	39	6·88	19·56	·05	9

The sum of all the F_2 segregations in which " white tip " appears is in good agreement with the expected 3 : 1 ratio. This is however misleading, as the heterogeneity between cultures significant (p smaller than ·01). This heterogeneity is mainly due to family I 14, which in its F_2 cultures shows an abnormally high incidence of " white tip " animals. As 5 of the 7 cultures segregated for the aberration, it is possible that one of the parents of Family I 14 had been homozygous for the factor. If this family is separated from the rest, a heterogeneity with a probability of ·05 is left.

The significant shortage of " white tip " beetles is due to low penetrance.

(c) *Orange head* (oh).—The head is covered with a white and orange pubescence, the white hair surrounding a patch of coloured hair. In the variation " orange head " the whole head is covered with orange hairs. The pubescence on the sides of the thorax and on the scutellum contains a higher proportion of orange hairs than the normal form.

The variation was noticed in one F_2 culture consisting of 11 animals, all of which showed it. It bred true in subsequent crosses.

(d) *Oval spot on the fifth abdominal segment* (o) (Fig. 10).—The bands which enclose the marginal spots on the fifth abdominal segment may decrease to such extent as to form two oval patches of white pubescence.
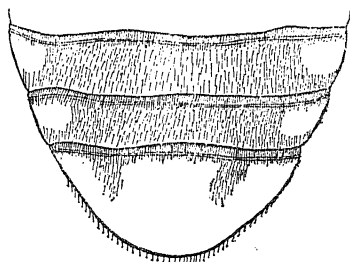
TEXT-FIG. 10. Abdomen of " Oval Spot ", Female

The character is fairly variable. It may have been missed in the original sample, because one culture consisted only of " oval " animals. Another

TABLE XX

F₂ segregations for " oval " (o)

Cross	+o	×	+o		χ^2	Heterogeneity χ^2	n	p
Phenotype	+o		o					
	♀	♂	♀	♂				
7 Cultures ..	68	52	16	20	·31	8·78	6	·2
Abnormal segregations	..	..	8	10	54·0			
,,	7	7	8	5	8·34			
Total ..	75	59	32	35	7·44	71·43	8	< ·01

culture gave a clear 1 : 1 segregation. Apart from those cultures the F_2 data are homogeneous giving reasonably good 3 : 1 ratios.

A similar variation was found in one family of population 1938 II.

TABLE XXI

F_2 segregations for " dexter " (d)

Cross	$+d$ $\times$ $+d$				χ^2
Phenotype	$+d$		d		
	♀	♂	♀	♂	
4 Cultures	36	46	12	4	4·38

(*e*) *Dexter* (*d*).—Because of the shortened and thickened legs, specially of the femur, this variation was called " dexter ". Besides the legs the eusternum of the pro- and mesosternum is altered in shape. The angle at the tip becomes more acute and the whole of the organ protrudes. As the character is variable, the abnormality of the eusternum being the most constant feature, there may be normal overlaps. There is a deficiency of abnormal males coupled with an excess of normal ones which suggests lowered penetrance in the males.

(*f*) *Truncated wing* (*T*) (Fig. 11).—In one culture of 43 individuals three males with wings similar to the truncated wing found in *Drosophila*

TEXT-FIG. 11. " Truncated Wing "

melanogaster were found. No further information is available as all the truncated males were sterile and the factor was not found again in the progeny of the normal brothers and sisters.

(*g*) *Cross vein in anal cell* (*an*).—In two cultures of one F_2 beetles with a deformed anal cell with cross vein, were found.

(*h*) *Cross vein in sector* (*se*).—In one family both in population 1938 I and II appeared animals with a cross vein in the distal part of the sector.

Population 1938 *II*—

(*a*) *Sex-linked lethal.*—In one fairly large family with 5 F_2 cultures a significant deficiency of males was found. The sex ratio in this family was 139 females to 93 males. The sex ratio in the F_2 of the rest of population 1938 II is 406 females to 403 males.

(b) *Hourglass* (h).—An area comparable to an hourglass is spared out on the fourth abdominal segment. It appeared in one culture of 21 animals of which 7 showed the variation.

The mutant breeds true, there is little variation in the manifestation and the viability is excellent.

Phenotypical variations—

Besides the aberrations from the wild type, which probably have a genetical basis, some variations were found which may be looked upon as phenotypic modifications. Under this term, which really only covers up our ignorance as regards the underlying embryological and genetical processes, may be summarised such variations which, among the limited number of off-spring raised, did not produce one of the original type. The character may have been determined genetically, but by a gene of such low penetrance that no aberrant animal could be expected unless the culture had been larger.

(a) *Gaping abdominal segment.*—During morphogenesis the two halves of the sternites grow out from both sides and subsequently join up. In this aberration the two halves have failed to do so. They do not touch and have developed a fringe of thick white pubescence. This type was found once.

(b) *Patchy abdomen.*—Instead of joining the half of the corresponding segment the growing sternite or tergite fuses with the other half of the following or preceding segment, leaving two free halves on either side. This disturbance of segmentation can be observed in the larvæ and followed up in pupa and adult. The same disturbance has been described in a number of insects. In *Drosophila melanogaster* a type like this occurs frequently, though we know little of its genetic behaviour. This aberration turned up in three independent cases, but was not recovered in later generations.

(c) *Fused antennæ.*—The two subterminal joints are fused into one. The animal was sterile.

(d) *Bubbly wing.*—Frequently adults are found in which the liquid is collected in the wing. Animals like this appear irregularly and are invariably sterile. They are mostly found in cultures in which the food has deteriorated badly.

Discussion

The three populations which have been described in this paper are of different origin and probably of different constitution. Two, population 1937 and population 1938 I, came from the inside of bales and must have been established from a small number of original invaders during a few generations. The third, population 1938 II, was collected from the outside of a pile of skins and was probably a beginning infection. There were several stray beetles and two clusters of larvæ, almost certainly derived from one batch of eggs.

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All three populations are polymorphic, due to some simple mendelian factors. This was used to determine the mating system in the colonies. In a former paper (Philip, 1938) data were given to show that mating was at random in population 1937 so far as the two pairs of allelomorphs $L-l$ and $B-b$ were concerned.

The method to ascertain random mating was to determine the ratio of homozygotes to heterozygotes. According to the Pearson-Hardy Law, the proportions expected are $u^2AA : 2uAa : aa$. In population 1937 the

TABLE XXIII a

Frequency of L/L and L/l animals

		Phenotype		Genotype		χ^2	n	p
		L	l	L/L	L/l			
Population 1937	Obs.	119	222	4·73	44·27			
	Exp.	118·35	222·65	5·21	43·79			
	χ^2	·003	·002	·46	·06			
Random mating						·525	1	·5
Population 1938 1	Obs.	84	153	·995	16·005			
	Exp.	93·3	143·7	1·83	15·17			
	χ^2	·927	·06	·386	·047			
Random mating						1·42	1	·2
Population 1938 II	Obs.	16	22	1	9			
	Exp.	15·71	22·29	1·32	8·68			
	χ^2	·003	·002	·079	·011			
Random mating						·094	1	·8
Total	Obs.	219	397	6·72	69·28			
	Exp.	217·37	399·63	8·24	67·76			
	χ^2	·012	·006	·290	·033			
Random mating						·321	1	·6

TABLE XXIII *b*

Frequency of B/B and B/b animals

		Phenotype		Genotype		χ^2	n	p
		B	*b*	B/B	B/*b*			
Population 1937	Obs.	96	56	15·95	37·97			
	Exp.	97·86	54·14	13·62	40·30			
	χ^2	·035	·064	·399	·135			
Random mating						·633	1	·4
Population 1938 I	Obs.	237	..	46	..			
	Exp.	..	..	..	..			
	χ^2	..	..	..	..			
Random mating						..	..	..
Population 1938 II	Obs.	30	8	3·99	7·01			
	Exp.	26·32	11·6	3·11	7·84			
	χ^2	·515	1·167	·249	·099			
Random mating						2·03	1	·1
Total	Obs.	363	64	65·94	44·98			
	Exp.	372·94	54·04	52·71	58·21			
	χ^2	·265	1·828	3·321	3·01			
Random mating						8·421	1	<·01

TABLE XXIII*c*

Frequency of A/A and A/a animals

		Phenotype		Genotype		χ^2	n	p
		A	a	A/A	A/a			
Population 1937	Obs.	?	?	?	?			
	Exp.	..	..	..	..			
	χ^2	..	..	..	..			
Random mating						..	..	..
Population 1938 I	Obs.	83	58	·946	15·054			
	Exp.	86·26	54·74	3·716	12·284			
	χ^2	·123	·194	1·078	·629			
Random mating						2·024	1	·1
Population 1938 II	Obs.	33	5	2·941	10·059			
	Exp.	31·31	6·69	5·331	7·669			
	χ^2	·091	·427	1·520	·745			
Random mating						2·783	1	·1
Total	Obs.	116	63	3·887	25·113			
	Exp.	113·4	65·6	7·1	21·9			
	χ^2	·176	·176	1·373	·447			
Random mating						2·098	1	·1

agreement between observation was very good. With some more data at our disposal than at the time of publication the χ^2 for L–*l* is ·52 and B–*b* is ·63.

The same tests repeated on the other two populations comprising 3 pairs of allelomorphs (L–*l*, B–*b* and A–*a*) confirmed the previous findings. Mating within the populations was at random. The test at the same time demonstrates that there cannot be a selection for any one of the genotypes responsible for the polymorphism. The relative frequencies of the different genes

are compared in Table XXIII. The allelomorphs L–*l* occur in almost exactly the same ratio in all three populations. B–*b* is represented in similar proportions in population 1937 and population 1938 II. But *b* is missing altogether in population 1938 I. The incidence of A–*a* in population 1937 is undetermined because the variation was only recognised in F_2 generation. Population 1938 II differs significantly from population 1938 I with regard to this factor.

From the fact that these colonies are started by only a few individuals, a wide range of variation in the ratios of different allelomorphs in different loci is to be expected. The degree of similarity in the frequency of L–*l* is therefore rather surprising. The test for random mating showed close agreement with the expectation. But the ratios in the single cultures vary greatly, as indicated by the high degree of heterogeneity in Table XXIII *a*. It will therefore be interesting to find out whether this result is due purely to chance or whether any special method of balance operates under field conditions.

The complete absence of *b* in population 1938 I and different proportions of A–*a* in population 1938 I and 1938 II are to be expected.

Taking all three populations together in the same test, it is possible to show that mating could not have been at random. As the proportions of L–*l* are so alike, no deviation from the hypothesis of random mating was found ($\chi^2 = \cdot 321$). The same applies to A–*a* ($\chi^2 = 2 \cdot 098$). Though significantly different in proportions, the number of animals used was too small to make any difference. Only for B–*b* χ^2 becomes 8·421, clearly demonstrating that the population could not have been uniform.

To detect evidences of random mating, as many loci as possible should be used for such a test.

The other variations which are responsible for the polymorphism found are a number of " genes " which affect the wing venation and are variable as regards expression, penetrance and dominance. They occur openly in one sample and not in the other. If the totals of the F_2 segregation are added together, differences between the three populations are found. The incidence of *r* is significantly higher in population 1937 than in the other two. " Extra vein ", though present in all three communities, is more numerous in population 1937. Population 1938 I and population 1938 II resemble each other more than population 1937. The incidence of " irregular cubitus " is alike in all groups. Whereas for " irregular cross vein ", population 1938 I differs more from population 1938 II than from population 1937 (Table XXIII).

E6   F

The relationship of the three populations given in diagrammatical manner can be roughly represented like this:

Population 1937 ←→ Population 1938 II ←→ Population 1938 I

i.e., Population 1937 resembles Population 1938 II. Then either of them resemble population 1938 I, though the 2 populations 1938 I and II have a certain amount in common.

Besides the factors responsible for the polymorphism in the F_1 and F_2 of 62 families, a number of more or less straightforward factors were isolated as described in the previous section.

As the populations under investigation were breeding units, derived from a limited number of ancestors, some of the mutants are fairly widespread within the community and therefore occur in more than one family.

The inbreeding experiment can give some information on the frequency of genes with known inheritance, if the single families are weighted according to the number of F_2 cultures raised and their respective size. Haldane has given a method for correcting data of this kind in a separate paper.

Because of the limitation of the breeding experiments, not all possible information is extracted from the populations. The degree to which they have been tested is given in Table XXII.

TABLE XXII

Population	No. of families	No. of families tested to 100%	% tested
1937 ..	32	15·57 ·	48·65
1938 I ..	22	17·15	77·27
1938 II ..	8	5·04	62·96
Sum ..	62	39·76	64·13

In Table XXIV the number of families in which the respective mutants occur is given.

The frequencies of the dominant allelomorph of some loci are calculated according to Haldane's method. These calculations are made on the assumption that the mutants give segregations of exactly 3 : 1 in heterozygote × heterozygote matings. Only those, which show an approximation and a 3 : 1 ratio have been calculated. The frequencies put down in the following table (XXV) are only limiting values.*

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* In case of the upper variations such a 'limiting value' has little sense and the tabulation has been omitted.

TABLE XXIV

No. of families segregating for mutants

Factor	r	ex	cu	cv	an	sc	w	sh	co	pl	2 sp	wt	o	d	Sex 1
Population 1937	20	5	2	5	..	..	10	2	1	1	1?	..	..	..	..
Population 1938 I	5	8	7	13	1	1	..	..	..	..	15	7	6	3	..
Population 1938 II	2	1	3	..	..	1	..	..	..	..	..	..	1	..	1

TABLE XXV

Frequencies of some dominant allelomorphs

	L	B	A	W	Sh	Co	Wt	O	D	2 Sp
Population 1937	·19	·61	?	·83 ±·05	·97 ±·02	·99 ±·01	1·00	1·00	1·00	1·00
Population 1938 I	·19	1·00	·35	1·00	1·00	1·00	·89 ±·03	·93 ±·03	·95 ±·02	49
Population 1938 II	·24	·54	·62	1·00	1·00	1·00	1·00	·92 ±·08		1·00

The fact that the majority of the factors with straightforward inheritance occur only in one population demonstrates the independence of the colonies.

In Table XXV the incidence of these factors in the total F_2 is given.

We have found in colonies of *Dermestes vulpinus* four types of variation:

(*a*) Factors contributing to the open polymorphism :—

> (1) Factors with straightforward mendelian inheritance widely spread, but subject to variations in frequency.

> (2) Factors with irregular inheritance, present in all populations, but not always segregating openly.

(*b*) Factors contributing to the hidden variability :—

> (1) Factors with straightforward mendelian inheritance, confined to one population with frequencies varying from 16%–1%.

> (2) Factors with irregular inheritance confined to one population.

The factors contributing to the polymorphism and those hidden under the surface do not differ by way of inheritance. There is possibly a difference in viability. Individuals homozygous for *sh* or *co* are very weak and already the heterozygote is possibly selected against. " White eye " was not found to be handicapped under culture conditions, but may not be so well equipped as the wild type beetle.

" White eye " has been found in several orders of insects without having been established as the " type " in any, which in itself is a demonstration of its disadvantage in the struggle for life.

The variability of *Dermestes vulpinus* described here is of the same type and of a similar order of magnitude as worked out in *Drosophila* species. This disposes of the view that the genes *Drosophila* especially *D. melanogaster* is exceptionally variable.

This work differs from the work on free-living populations of *Drosophila* species by the actual observation of breeding groups, which stay in a minute locality for a number of generations. The polymorphism found is possibly the result of this.

Conditions of selections and migration are somewhat abnormal under the storehouse conditions. Therefore no further conclusion on the actual genetical behaviour of polymorphic species can be advanced before this animal has been studied in its natural habitat.

Summary

Three populations of *Dermestes vulpinus* were investigated for genetical variability.

Polymorphism not previously described was found to be due to three pairs of allelomorphs, behaving as simple mendelian factors, and a number of factors, affecting the venation of the wing showing various degrees of realisation.

Seven factors with simple mendelian inheritance were isolated in F_2 and F_3 generation.

One factor, copying a secondary sexual character of the other sub-group of Dermestidæ, behaved as an autosomal recessive with sex-limited action.

One sex-linked lethal was found.

Mating within each of the populations was at random. But taking the three populations as an entity, significant disagreement with the hypothesis of random mating was revealed.

A comparison showed the three colonies to be largely independent.

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SOME STUDIES ON THE PHYSIOLOGY OF *CYTOSPORA SACCHARI* BUTL., THE CAUSAL FUNGUS OF STEM CANKER DISEASE OF SUGARCANE

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CONTENTS

I. *Introductory*

IN the previous publication (1938) it has been shown that *Cytospora sacchari* Butl. is parasitic on sugarcane under Punjab conditions. Studies of the causal fungus under conditions of artificial culture were, therefore, carried on with a view to a thorough understanding of its physiology. The present paper embodies the results of these investigations conducted during the last two years.

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II. Experimental

A. Material and methods.—

The fungus *Cytospora sacchari* was isolated from diseased canes of variety Co. 313. It was purified by monosporal culturing.

The following were the media used for growing the fungus :—

1.	Oat-meal agar ..	Oat-meal 50 gms.; agar agar 20 gms.; distilled water 1,000 c.c.
2.	Richards' agar ..	Magnesium sulphate 2·5 gms.; potassium dihydrogen phosphate 5 gms.; potassium nitrate 10 gms.; sucrose 50 gms.; agar agar 20 gms.; distilled water 1,000 c.c.
3.	Brown's agar	Asparagin 2 gms.; magnesium sulphate 0·75 gms.; glucose 2 gms.; potassium phosphate 1·25 gms.; agar agar 15 gms.; distilled water 1,000 c.c.
4.	Brown's starch agar ..	Medium 3 *plus* potato starch 10 gms.
5.	Nutrient glucose agar	Glucose 20 gms.; peptone 10 gms.; sodium chloride 5 gms.; extract of meat 4 gms.; agar agar 15 gms.; distilled water 1,000 c.c.
6.	Gur agar	Gur (raw sugar) 30 gms.; magnesium sulphate 1 gm.; potassium monohydrogen phosphate 1 gm.; iron perchloride traces; agar agar 20 gms.; distilled water 1,000 c.c.

B. Linear growth rate.—

(1) *Effect of depth of medium.*—For the purpose of studying the effect of depth of medium on the linear colony growth of the fungus Richards' agar was selected. Petri dishes of uniform size, each 4·5 inches in diameter, were sterilised and divided in five sets of four each. 10 c.c. of the medium was poured in every dish of the first set, 20 c.c. in the second, 30 c.c. in the third, 40 c.c. in the fourth and 50 c.c. in the fifth set. Thus the depth of the medium in the petri dishes was proportional to the amount of the medium poured. These were then ·inoculated with the fungus and placed in the incubator at a temperature of 24° C. Observations on the linear growth of the colony were recorded after seven days. The results are shown·in Fig. 1.
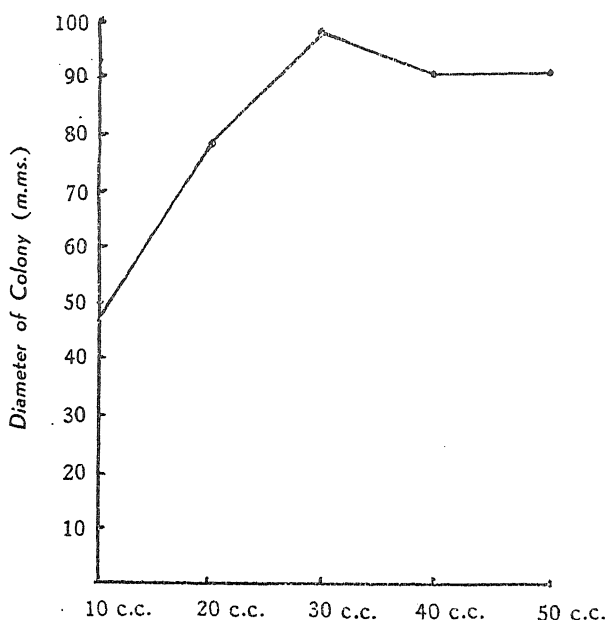
FIG. 1

Colony diameter of *C. sacchari* on different depths of Richards' agar after 7 days' growth at 24° C.

(*Cytospora sacchari*)

It is clear that the colony growth increases with the depth of the medium, the maximum growth being at 30 c.c. At 40 c.c. and 50 c.c. the growth is slightly less than at 30 c.c. On account of the difference in the linear colony growth of the fungus at different depths of the medium care was taken to have uniform depth of the medium in all future experiments.

(2) *Effect of medium.*—The fungus was grown on six different media at 30° C. and the colony growth was measured after four days. The results are presented in Fig. 2.

It is clear that gur agar, oat-meal agar and Richards' agar are better for the linear growth of the fungus than nutrient glucose agar, Brown's agar and Brown's starch agar.

(3) *Effect of concentration of medium.*—The fungus was grown on different concentrations of Richards' agar at 26° C. N/1 Richards' agar means normal Richards' agar, N/2 means the nutrients of the medium contained in two litres of water and 2 N means the nutrients of the medium contained in half a litre of water and so on. Observations were recorded after seven days and are given in Fig. 3.
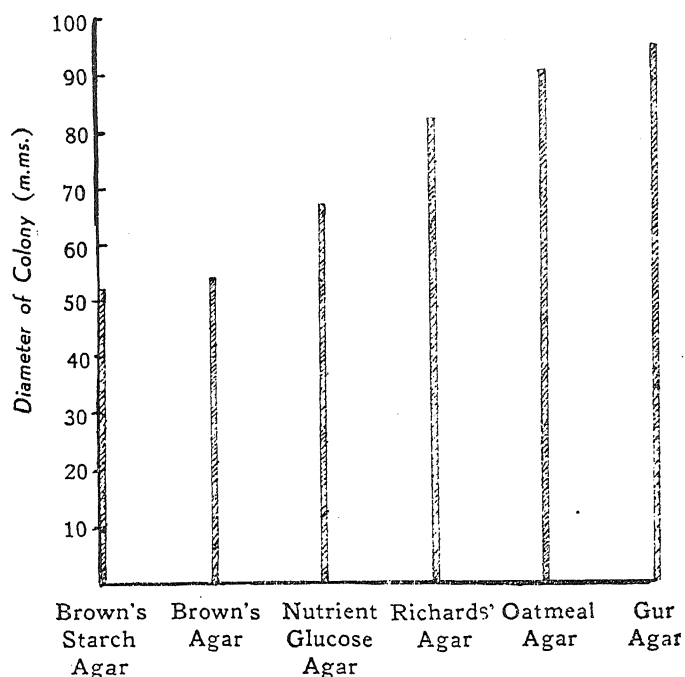
Fig. 2

Colony diameter of *C. sacchari* on different culture media
after 4 days' growth at 30° C.
(*Cytospora sacchari*)
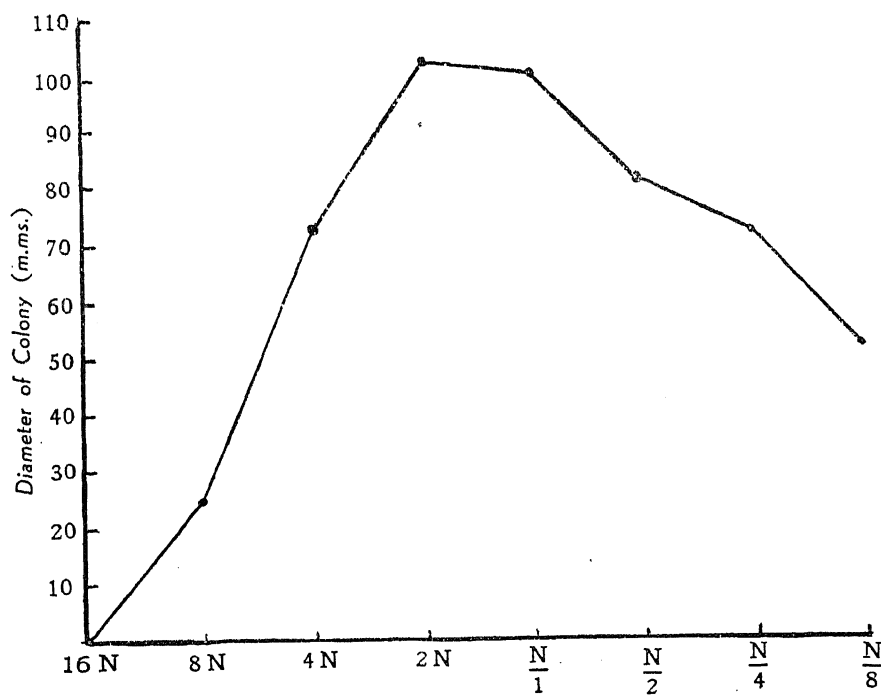
Fig. 3

Colony diamter of *C. sacchari* on different concentrations of Richards' agar
after 7 days' growth at 26° C.
(*Cytospora sacchari*)

It is clear that the maximum growth of the fungus is on N/1 and 2 N Richards' agar and it declines both with the increase and decrease of the concentration of the medium, the fall being more rapid with the increase of the concentration of the medium than with the decrease. At 16 N there is no growth.

(4) *Effect of nutrients.*—The fungus was grown at 25° C. on normal Richards' agar and on Richards' agar in which magnesium sulphate, potassium dihydrogen phosphate, potassium nitrate or sucrose was lacking. Observations were recorded after six days and are presented in Fig. 4.

| pH | 5·0 | 5·0 | 4·8 | 6·6 | 5·0 |
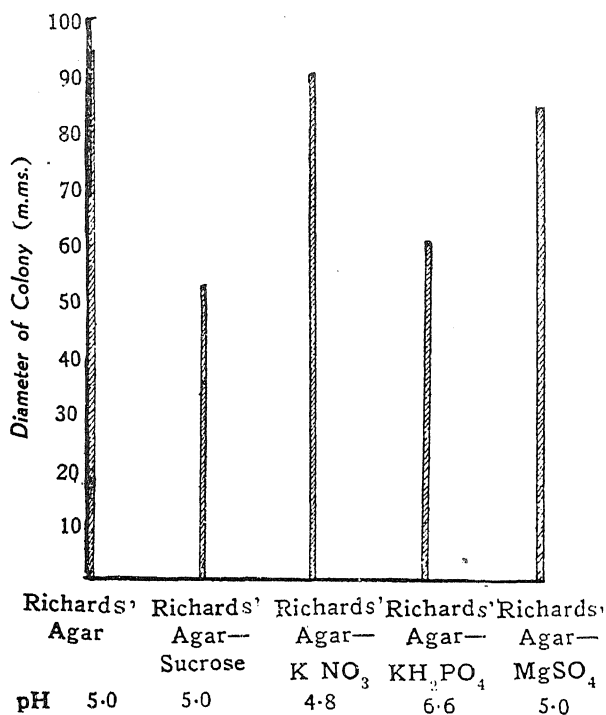
FIG. 4

Colony diameter of *C. sacchari* on Richards' agar
Normal and lacking in different constitutents after 6 days' growth at 25° C.
(*Cytospora sacchari*)

It will be seen that when potassium nitrate is lacking the growth of the fungus is almost the same as on normal Richards' agar and that when magnesium sulphate lacks growth is slightly less than on normal Richards' agar. But when sucrose or potassium dihydrogen phosphate is missing the growth falls considerably, being only a little over half of that on normal Richards' agar.

Whether the effect produced by the absence of the different nutrients in the medium was due to the lack of a certain kind of nourishment or due to the changed hydrogen-ion concentration was further determined. All the media were prepared as in the last experiment and all of them were adjusted to pH 5·0. These were inoculated with the fungus and the dishes kept at 25° C. Observations were recorded after six days and are presented in Fig. 5.
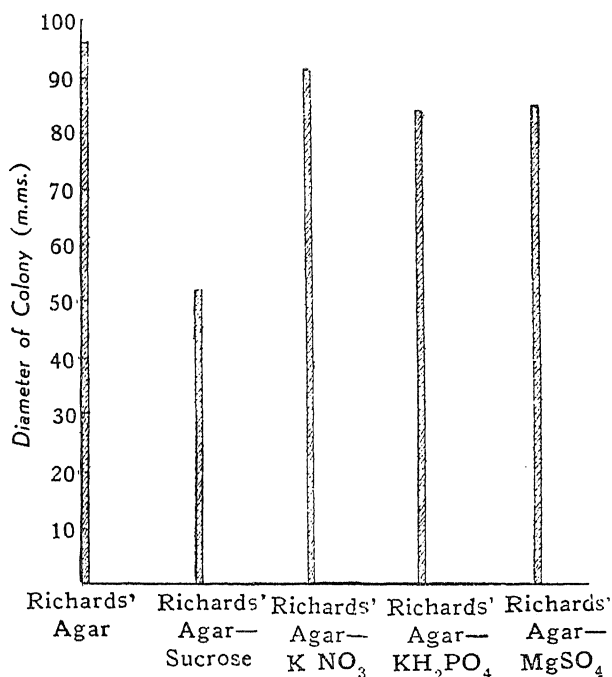
FIG. 5

Colony diameter of *C. sacchari* on Richards' agar
Normal and lacking in different constituents and adjusted to pH 5·0, after 6 days' growth at 25° C.
(*Cytospora sacchari*)

The results show that when the pH of all the media was adjusted to 5·0 there was not much difference in the colony growth of the fungus in any of the media except in the case where sucrose was eliminated.

(5) *Effect of different sugars.*—For the purpose of studying the effect of different sugars on the linear colony growth of the fungus Richards' agar was selected. Richards' agar without any sugar, with the normal quantity of sucrose and with the same quantity of glucose or maltose or lactose was prepared and inoculated with the fungus and kept at 26° C. Observations were recorded after six days and are presented in Fig. 6.
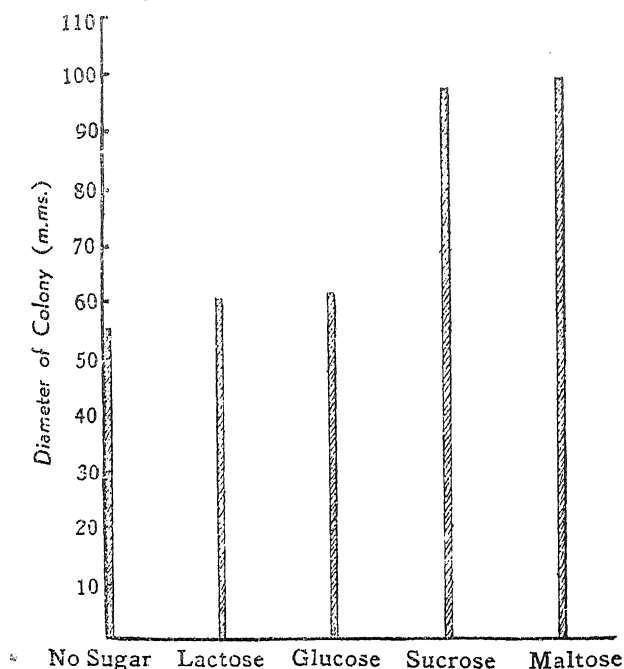
FIG. 6

Colony diameter of *C. sacchari* on Richards' agar
Containing different sugars and no sugar after 6 days' growth at 26° C.
(*Cytospora sacchari*)

It will be seen that growth in normal Richards' agar, *i.e.*, when it contains sugar in the form of sucrose and in Richards' agar when maltose is substituted for sucrose is almost equal. When glucose or lactose is substituted for sucrose or when no sugar is added the growth falls considerably and comes down to a little over half as much as that on normal Richards' agar. The interesting point to note is that the growth is almost the same when glucose is substituted for sucrose or when no sugar is added at all; thus showing that sugar in the form of glucose does not play any important part in the linear colony growth of the fungus. Similarly lactose seems to play little part.

(6) *Effect of different quantities of sucrose.*—Nine grades of Richards' agar differing in the amount of sucrose content varying from zero gram to 800 grams per litre were prepared and were inoculated with the fungus. The dishes were kept at 24° C. Colony growth was measured after six days and the results are presented in Fig. 7.

It is clear that the growth gradually increases with the amount of sugar till it reaches its maximum when the amount of sugar is 200 grams or 400 grams per litre.
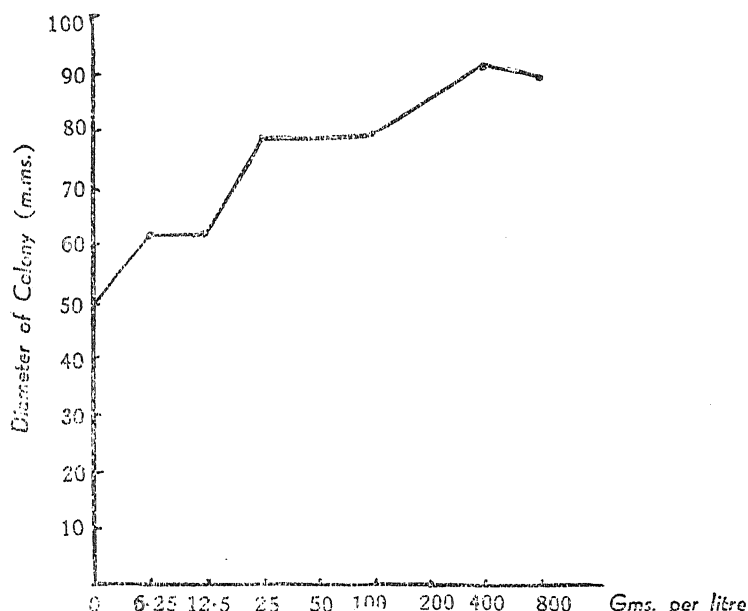
FIG. 7

Colony diameter of *C. sacchari* on Richards' agar
Containing different quantities of sucrose after 6 days' growth at 24° C.
(*Cytospora sacchari*)

(7) *Effect of temperature* —The fungus was grown on oatmeal agar and Richards' agar at temperatures varying from 5–40° C. Observations were recorded after four days on oatmeal agar and after five days on Richards' agar. The results are given in Fig. 8.
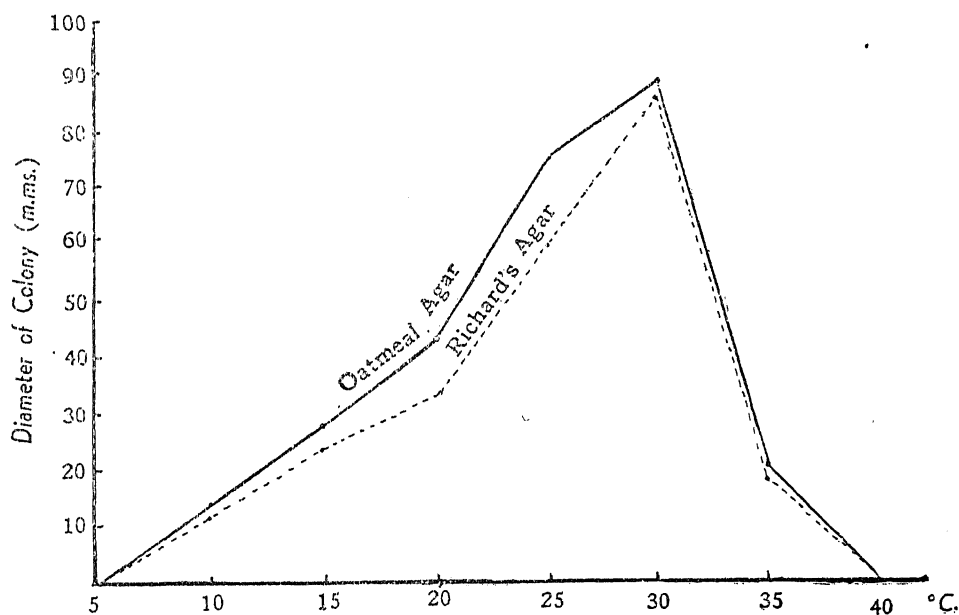
FIG. 8

Effect of temperature on colony diameter of *C. sacchari*
after 4 and 5 days' growth on oatmeal agar and Richards' agar
(*Cytospora sacchari*)

It is clear that the optimum temperature for the growth of the fungus on both the media is 30° C., the maximum being between 35° and 40° C. and the minimum below 15° C.

(8) *Effect of hydrogen-ion concentration.*—The effect of hydrogen-ion concentration on the colony growth was studied on Richards' agar at 30° C. Various pH ranges of the medium were adjusted by the addition of normal hydrochloric acid or normal sodium hydroxide and the resulting pH's were determined colorimetrically by Clark's (1923) method. Colony growth of the fungus was measured after six days. The results are given in Fig. 9.
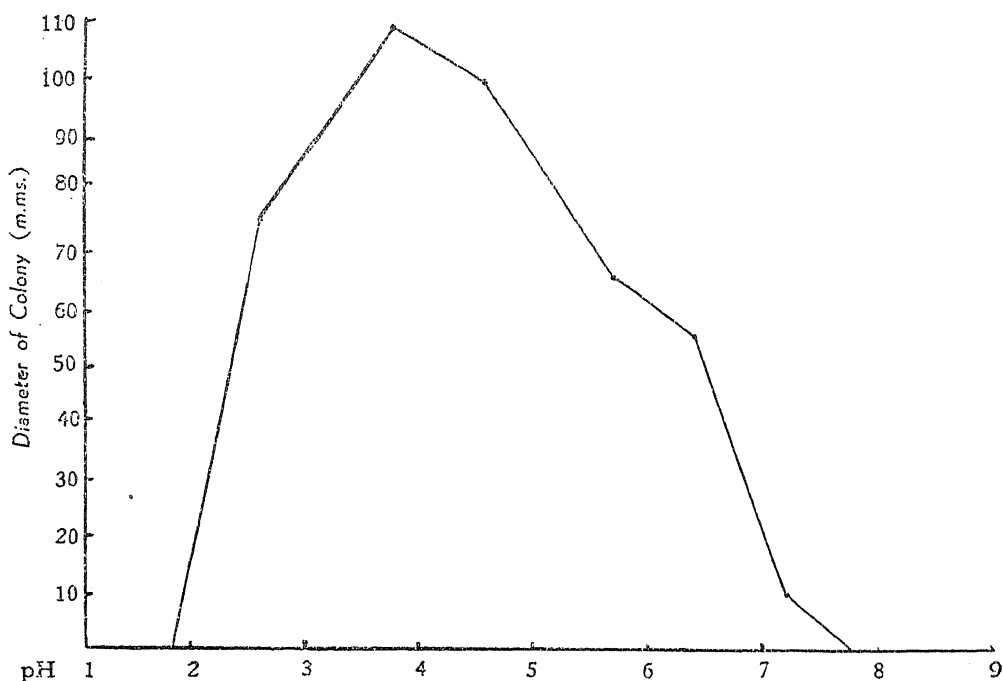
FIG. 9

Effect of pH on colony diameter of *C. sacchari*
after 6 days' growth at 30° C.

(*Cytospora sacchari*)

It will be seen that the maximum growth of the fungus is at pH 3·8. On the acid side growth stops at pH 1·8. At the neutral point the growth is negligible and on the alkaline side the growth stops at pH 7·8.

C. *Pycnidia formation.*—

The effects of the medium, concentration of the medium, depth of the medium, different sugars, different amounts of sucrose, light and temperature on the formation of pycnidia of this fungus have been studied in some detail and are described below.

(1) *Effect of medium.*—The fungus was grown on six different media at 30° C. and the observations on the formation of pycnidia were recorded after eight days, eighteen days and thirty days. The results obtained are given in Table I.

TABLE I

Effect of medium on the formation of pycnidia at 30° C.

Medium	Formation of pycnidia		
	After 8 days	After 18 days	After 30 days
Oat-meal agar ..	0	0	Moderate
Gur agar	0	1–3 Pycnidia	,,
Richards' agar	0	0	0
Nutrient glucose agar ..	0	0	0
Brown's agar	0	0	0
Brown's starch agar ..	0	0	0

Pycnidia were first observed on gur agar after eighteen days' growth and were later observed on oat-meal agar but no pycnidia were formed even after thirty-five days' growth on Richards' agar, nutrient glucose agar, Brown's agar and Brown's starch agar.

(2) *Effect of depth of medium.*—To study the effect of depth of a medium on the formation of pycnidia oat-meal agar was selected. The medium was poured in petri dishes in such a way that the depth of the medium was graded from very shallow on one side to very deep on the other. The petri dishes before pouring the medium were marked with ink at the bottom so as to represent four distinct regions, two on either side of the inoculum which represented the centre of the plate. Observations were recorded after forty days' growth of the fungus at 30° C. and it was found that the formation of pycnidia was distinctly more pronounced on the deep medium whereas the number of pycnidia diminished with the shallowness of the medium and very few pycnidia were formed where the medium was very shallow.

(3) *Effect of concentration of medium.*—Oat-meal agar of different concentrations—2 N representing that the constituents are in 500 c.c. of water and N/2 representing that the constituents are in 2,000 c.c. of water—was prepared. Observations on the formation of pycnidia were recorded after twenty-nine days' growth at 27° C. The results are given in Table II.

TABLE II

Effect of concentration of oat-meal agar on the formation of pycnidia after twenty-nine days' growth at 27° C.

Concentration of medium	Formation of pycnidia
4 N	Fair
2 N	Fair
N/1	Moderate
N/2	Moderate
N/4	Scanty
N/8	Scanty

It is clear that the number of pycnidia produced varies in direct proportion to the concentration of the medium.

(4) *Effect of different amounts of sucrose.*—As the pycnidia could not be produced on Richards' agar therefore oat-meal agar was selected in order to study the effect of different amounts of sucrose on the formation of pycnidia. Six different sets of oat-meal agar, in which the quantity of sucrose varied from 6·25–200 grams per litre, were prepared. Observations on the formation of pycnidia were recorded after thirty-seven days' growth at 26° C. and are given in Table III.

TABLE III

Effect of different amounts of sucrose on the formation of pycnidia after thirty-seven days' growth at 26° C.

Quantity of sucrose	Formation of pycnidia
Grams	
200	Fair
100	Fair
50	Moderate
25	Moderate
12·5	Scanty
6·25	Scanty
0	Scanty

It is clear that the number of pycnidia formed is in direct proportion to the amount of sucrose present in the medium.

(5) *Effect of different sugars.*—To study the effect of different sugars on the formation of pycnidia Richards' agar was selected and maltose, glucose and lactose were individually substituted for sucrose and normal Richards' agar as well as Richards' agar without sucrose were also included. Observations were recorded after thirteen days' growth at 26° C. The results are given in Table IV.

TABLE IV

Effect of different sugars on the formation of pycnidia
after thirteen days' growth at 26° C.

Medium	Formation of pycnidia
Richards' agar containing maltose	Fair
Richards' agar containing sucrose	0
Richards' agar containing sucrose	0
Richards' agar containing glucose	0
Richards' agar without sugar	0

It will be seen that the pycnidia formed abundantly on Richards' agar containing maltose and were absent in the media containing sucrose or lactose or glucose or no sugar. The interesting point to note is that on Richards' agar where pycnidia formation does not ordinarily take place these were observed after thirteen days' growth when maltose in place of sucrose was substituted.

(6) *Effect of light.*—An experiment conducted with oat-meal agar to study the effect of light and darkness on the formation of pycnidia showed that the fungus forms pycnidia more readily in light than in darkness. Further the number of pycnidia was much less on the side of the dishes away from light than on the side towards light.

(7) *Effect of temperature.*—The effect of temperature on the formation of pycnidia was studied on oat-meal agar, Richards' agar and pieces of cane stem. Observations were recorded after twenty-eight days' growth and the results are given in Table V.

TABLE V

*Effect of temperature on the formation of pycnidia
after twenty-eight days' growth*

Temperature in °C.	Formation of pycnidia on		
	Oat-meal agar	Richards' agar	Cane setts
5	No growth	No growth	No growth
15	0	0	Scanty
20	2–3 Pycnidia	0	Moderate
25	4–5 Pycnidia	0	Fair
30	25–30 Pycnidia	0	Moderate
35	0	0	0
40	No growth	No growth	No growth

It will be seen that on oat-meal agar pycnidia were formed at 20° C., 25° C. and 30° C. only and the greatest number of pycnidia formed was at 30° C. No pycnidia were formed on Richards' agar at any of the temperatures while on cane stems pycnidia were formed at 15° C., 20° C., 25° C., and 30° C., the greatest number being formed at 25° C.

D. *Formation of aerial mycelium.*—

The formation of aerial mycelium was studied on different media under a variety of conditions. The amount of aerial mycelium on different media is given in Table VI from which it will be seen that oat-meal agar and

TABLE VI

*Effect of medium on the formation of aerial mycelium
after four days' growth at 30° C.*

Medium	Formation of aerial mycelium
Oat-meal agar	Fair
Brown's starch agar	Scanty
Nutrient glucose agar	Fair
Gur agar	Moderate
Brown's agar	Scanty
Richards' agar	Scanty

nutrient glucose agar are the best for the formation of aerial mycelium and Brown's starch agar, Brown's agar and Richards' agar produce very scanty aerial mycelium while gur agar stands intermediate in this respect.

Out of the environmental factors temperature and hydrogen-ion concentration have been found to have some effect on the formation of aerial mycelium. The effect of *temperature* on the formation of aerial mycelium is shown in Table VII from which it will be seen that aerial mycelium is produced only at 20° C., 25° C. and 30° C. It is more at 25° C. and 30° C. than at 20° C.

TABLE VII

Effect of temperature on the formation of aerial mycelium
after five days' growth

Temperature in °C.	Formation of aerial mycelium on	
	Richards' agar	Oat-meal agar
5	No growth	No growth
15	0	0
20	0	Scanty
25	Trace	Moderate
30	Trace	Moderate
35	0	0
40	No growth	No growth

The effect of *hydrogen-ion concentration* on the formation of aerial mycelium on Richards' agar at 30° C. is given in Table VIII from which it will be seen that at all hydrogen-ion concentrations where the growth of the fungus can take place there is a fair amount of aerial mycelium.

TABLE VIII

Effect of hydrogen-ion concentration on the formation of
aerial mycelium after fifteen days' growth

Hydrogen-ion concentration	Formation of aerial mycelium
1·8	No growth
3·8	Scanty
4·8	Scanty
5·8	Moderate
6·4	Moderate
7·0	Moderate
7·8	No growth
8·5	No growth
9·6	No growth

E. *Colony colour.—*

The colour of the colonies of the fungus produced on different media under a variety of conditions was recorded and it was found that the colour of the colonies remained white on all the media under different environmental conditions.

III. Discussion

The investigations embodied in the text have revealed that the fungus *Cytospora sacchari* is one of the most interesting parasitic fungi recorded in India. The points which make it interesting are:—

1. Its growth relation to hydrogen-ion concentration.

2. Its response to different sugars.

As regards the first point it has been shown that the fungus produces its maximum growth at pH 3·8 and at about the neutral point its colony growth is practically insignificant. This finding is of very great significance from the practical point of view. As the Punjab soils are generally alkaline in reaction with a pH about 7·0–8·5 it is evident that this fungus would not be able to grow in most of the Punjab soils and thus the possibility of infection of canes by this fungus from the soil will be eliminated. This point has been borne out by investigations which will be recorded in a later communication. Apart from the practical point referred to above this

behaviour of the fungus is of great academic interest and the fungus can be included in the college curriculum for demonstration to students of Botany and Mycology.

As regards the second point it has been shown that when sucrose is omitted from Richards' agar the colony growth of the fungus falls by about forty per cent. If lactose or glucose is substituted for sucrose in Richards' agar the colony growth remains almost the same as on the medium without sucrose. But when maltose is substituted the growth becomes almost normal. It is thus clear that sucrose and maltose play a great part in the linear colony growth of the fungus and lactose and glucose do not seem to play any part at all. That sucrose plays a considerable part in the colony growth of the fungus is further evident from the fact that the colony growth of the fungus on synthetic media such as Brown's agar, Brown's starch agar, and nutrient glucose agar is much less than on Richards' agar. On a natural medium, *viz.*, oat-meal agar the growth is the same as on Richards' agar although it does not contain sucrose. As the composition of oat-meal is of a complex nature it is difficult to explain this behaviour of the fungus from a purely scientific point of view. But it seems probable that this is due to the richness of the medium.

IV. Summary

A study of the fungus as regards its colony growth, formation of pycnidia and aerial mycelium has been made in artificial culture media under a variety of conditions. The important results obtained are:—

1. For linear growth of the fungus gur agar, oat-meal agar and Richards' agar are better than nutrient glucose agar, Brown's agar and Brown's starch agar.

2. The maximum growth of the fungus takes place on normal and on twice as concentrated Richards' agar. Above and below these concentrations the growth falls.

3. Elimination of sucrose from Richards' agar reduces the growth of the fungus, but the elimination of potassium nitrate, potassium dihydrogen phosphate or magnesium sulphate from the medium individually does not materially affect the growth if after elimination pH of the medium is adjusted to pH 5·0.

4. When sucrose is omitted from Richards' agar or when glucose or lactose is substituted for sucrose the growth of the fungus falls but when maltose is substituted for sucrose the growth remains unaffected. The growth gradually increases with the amount of sucrose in Richards' agar till it reaches its maximum when the amount of sucrose is 200 grams per litre.

5. The optimum temperature for the growth of the fungus is about 30° C., the maximum being between 35 and 40° C. and the minimum below 15° C.

6. The maximum growth of the fungus is at pH 3·8. On the acid side growth stops at pH 1·8 and on the alkaline side at pH 7·8. The importance of this interesting behaviour of the fungus in relation to pH has been discussed.

7. The fungus forms pycnidia on gur agar and oat-meal agar but none on other media within thirty-five days. The number of pycnidia produced on oat-meal agar varies in direct proportion to the concentration of the medium as well as to the amount of sucrose added in oat-meal agar.

8. Light as well as maltose favour the formation of pycnidia.

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STRUCTURE AND FUNCTION OF THE NIDAMENTAL GLAND OF *CHILOSCYLLIUM GRISEUM* (MULL. AND HENLE)

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CONTENTS

Introduction

This piece of research on the nidamental gland of *Chiloscyllium griseum* was undertaken as a preliminary to a study of the structure and function of the gland in some of the common Elasmobranchs found in Madras. *Chiloscyllium*, a typically oviparous form, was chosen in the first instance on account

of its large nidamental or shell glands. They are best developed in the female of oviparous forms and generally very much less in the viviparous Elasmobranchs. Sometimes they are even totally absent.

Previous History

These glands were known as early as the days of Aristotle, and were called in the Ray as ' Bruste of Aristotle '.

Muller (1843) (as quoted by Borcea, 1905) remarks that there are great differences in the conformation of these glands which serve to secrete the shell of the egg. He says, that the gland is scarcely indicated in *Torpedo*, where no egg case is secreted; and it is doubtful whether it even exists in *Lamna*. But in oviparous rays, chimæras, etc., they are well developed. Muller also describes the various modifications in shape of the gland, met with in Squales.

Cuvier (1846) (as quoted by Borcea, 1905) describes the gland as a dilation of the oviduct, enveloping in its walls a glandular body composed of tubes. The secreted ' humour ' produces the shell of the egg, and the form of the shell is doubtless the same as the glandular surface.

Leydig (1852) considers the gland as situated outside the mucosa of the oviduct. The secretion, he says, is in the form of spiral filaments in two longitudinal grooves situated below the reunion of the two halves of the gland. The glands consist of tubules with fat globule contents (apparently, as Widakowich says, he took the granules of shell secretion for fat).

Martin St. Ange (1856) (as quoted by Borcea, 1904) describes the nidamental gland of the *Mustelus vulgaris*. This author believes that at the outset the shell secreted by the nidamental gland is placed in a manner intimate to the vitelline membrane, and as the egg travels along the oviduct, it detaches itself little by little from the vitelline sphere by the penetration of a notable quantity of albuminous liquid " par endosmose ", when the fœtus is at the point of birth. This liquid fulfils the function of the amnion of higher vertebrates.

Vogt and Pappenheim (1859) (as quoted by Borcea, 1904) worked on the nidamental gland of *Raia clavata*. They describe three zones,—a longitudinal, an oblique and a transversely striated one. The lower part of the gland has a zone larger than the preceding one which has a velvety surface.

Bruch (1860) remarks that the nidamental gland varies according to the species and the period of the year.

Gerbe (1872) (as quoted by Borcea, 1904) was the first to observe that the nidamental gland not only secretes the shell but also the albumen. Before him it was believed that the albumen was secreted in the cranial

oviduct. The production of albumen and shell, he says, is almost simultaneous.

Perravex (1884) described the nidamental glands of *Scyllium canicula* and *Scyllium catulus*. The stratified structure of the shell, he says, is due to the disposition of the transverse plates (referring to the lamellæ) found in the middle region. According to him, the gland cells do not contain fat droplets but secretory granules. He aims at describing the origin of the shell, without support of anatomical evidence. Perravex does not distinguish the different kinds of shell tubules.

Henneguy (1893) studied the histology of the albumen producing and the shell producing regions of the nidamental glands of *Scyllium canicula*. He describes large prismatic cells in the cranial part between which are inserted very narrow ciliated cells with central nuclei. The cells of the albumen producing region are transparent and filled with a fine protoplasmic reticulum and the big cells of the shell producing region are crammed with refringent granulations, insoluble in potash, and strongly coloured by Methyl green. He observed the narrow intermediate zone of mucous glands between the albumen and shell producing regions but thinks they are partly responsible for the secretion of the shell.

Disselhorst 1904 (as quoted by Widakowich, 1906) describes the nidamental glands of *Raia*.

Garman 1885 (as quoted by Widakowich, 1906) found in *Chlamydoselachus* which he took for a viviparous form a nidamental organ. He describes it and also gives a figure.

Borcea (1904) : His paper is quite a long one, dealing with the whole reproductive system of Elasmobranchs. He describes with diagrams the nidamental glands of *Scyllium canicula* and *Scyllium catulus*. He observes the three sets of glands which he names according to the nature of their respective secretions as albumen glands, mucous glands, and shell secreting glands. He is of opinion that among Elasmobranchs there are variations in the nature of the albumen secreted by the 1st zone of the nidamental gland. He compares his observations with those of Tarchanow and Lataste (1889) on the albumen of bird's eggs. As regards the formation of the egg case, Borcea (1904) is of the same opinion as Perravex, and considers the arrival of the egg usually necessary though other stimuli can incite the gland to function. He also deals with the secretion being deposited in layers by the help of the lamellæ, though he does not mention anything about the " zottchen " or " tufts " of Widakowich. Borcea ends his paper by touching lightly upon the development and vascularisation of the nidamental gland.

Widakowich (1906) published a paper on the "Structure and function of the nidamental organs of *Scyllium canicula*". Here we get a detailed and critical study of the structure and function of the gland. Widakowich was fortunate enough to find that three out of the forty specimens examined by him contained in their oviducts eggs with shells incompletely developed; which means that the egg case was just being formed. It was, therefore, comparatively easy for him to distinguish the different types of secretion and the mode of shell formation. He distinguishes four sets of glands, *viz.*, albumen glands, shell glands, intermediate large mucous glands and a set of smaller mucous glands lying in the inside of the organ at the caudal pole. The shell secreting glands, he divides, into two groups, one which secretes the filaments forming the cranial part of egg case and the other which secretes those emerging from the caudal end of egg case. According to him the major portion of the nidamental organ consists of small tubular shell glands secreting the shell matrix. He also describes the structure of the egg case. The plastic secretions according to him are rolled together into layers of suitable thickness by the help of the lamellæ and the egg gets covered with as many layers of secretion as there are inter-lamellar spaces.

No work has been done on the nidamental glands of any of the Indian species of Elasmobranchs and *Chiloscyllium griseum* is the first of its kind undertaken for this investigation.

Material and Methods

It was found very difficult to get specimens of suitable size and maturity. Specimens measuring less than 65 cm. were found immature and the glands in these were very imperfectly developed, if at all. However, six fully mature live specimens ranging in their size from 65–75 cm. were obtained in which the nidamental glands were found in a highly developed condition useful for the study of secretory activity. These were opened and the nidamental glands removed and fixed immediately in Nassanov, DaFano and other cytological fixatives for the demonstration of golgi. The results will form the subject-matter for another paper. Corrosive acetic formol proved the best fixative for histological study of the gland and material fixed in this gave very little trouble in cutting. This fixative was also found suitable for mucus test with Thionin blue. Other fixatives used were Bouin, Formalin 5%, Zenker formol, and Carnoy. The last was very useful for bringing out the nuclei. Bouin's fluid was helpful in demonstrating the general structure, but material fixed in this gave considerable trouble when sectioning. The picric acid had to be washed out completely with several changes of lithium carbonate in 70% alcohol before grading up.

In spite of this the material became quite brittle and quick penetration of paraffin was impossible in case of material fixed in Bouin's, with the result that I found it very difficult to obtain entire sections of the whole nidamental organ. The tough outermost covering of the oviduct and the nidamental organ was another factor that made section cutting very tedious ; for, sections crumbled and broke off before the knife at this point. Occasional immersions of the cut surface of the block in ice lessened to some extent this difficulty.

Solutions containing $HgCl_2$ or acetic acid, entirely destroy the early stages of albumen formation. Zenker's formol followed by Iron hæmatoxylin proved most suitable for demonstrating albumen formation. Complete sections of the nidamental gland, 8–10 microns thick, as well as sections of small bits of the gland 5–6 microns thick, were taken for histological study. The material, after grading up in alcohols was given two changes of Cedar-wood oil, 3–6 days, and left in the paraffin bath for an equal number of days. 58° paraffin was used. Smearing of a little soft paraffin on the upper and lower edges of the block enabled me to get good ribbons.

Stains used.—Iron Hæmetoxylin was found good for nuclear study especially after Carnoy. Counter-staining with van Gieson was helpful in differentiating the two sets of shell secreting glands. The part of the cranial zone just below the large mucous glands remained jet black, while the rest of the shell secreting tubules took a brownish-yellow colour. The cilia too appeared prominent as they were stained pink. The intermediate zone of mucous cells and those of the caudal pole were stained purple by thionin. The caudal mucous cells did not stain as intensely with thionin as the mucous cells of the intermediate zone. Mucicarmine was also used to demonstrate mucin. Mallory's triple stain served very well for general diffe-rentiation of tissues and the layers of the egg case. Keratin (outermost and innermost layers of egg case) stained in shades of red while the middle zone as well as the albumen, forming a thin coating to the inner wall of capsule, were blue.

General Structure of the Nidamental Gland

In the mature forms the nidamental glands appear as two solid structures situated 6·2 cm. below the common oviducal opening (Pl. V, Fig. 1). When the fish is slit open from the ventral side the nidamental glands lie flat attach-ed to the dorsal side by the highly vascular mesovarium. Often there is a twist in the reproductive system specially at the junction of the caudal oviduct with the uterus.

In the specimens examined both the glands were equally well developed. They are pear-shaped structures, broad anteriorly, tapering down below, and

B1 *a*

merging into the caudal oviduct. The swollen cranial portion measured
2·5 cm. in length and 3·75 cm. in breadth and the caudal tapering posterior
part 2·5 cm. in length. To the naked eye the nidamental organ consists
of two parts of different colours; a flesh-pink anterior zone and an opaque
white portion which occupies the rest of the cranial portion of the gland. The
latter portion is incompletely divided into right and left halves by a slight
external groove lying slightly on one side of the gland, to the right or left as
the case may be. This white portion extends down into the caudal pole for
an inch or more, after which the caudal oviduct starts. When slit open
along the dorsal side (Text-Fig. 1 a) the inner aspect of the gland shows the
three zones. The inside of the first zone is traversed by shallow grooves
with alternating ridges. The second or middle zone is the broadest and is
characterised by very delicate lamellæ with slightly wavy tops. With this
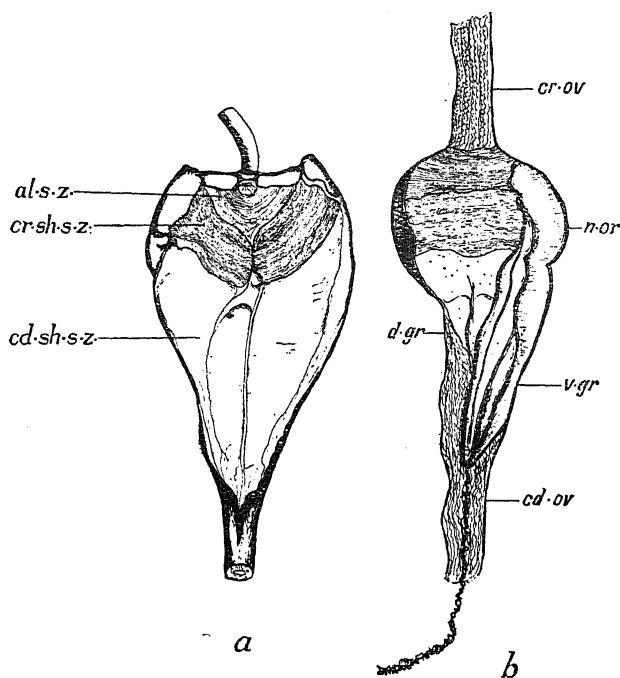
TEXT-FIG. 1

zone the anterior swollen part ends. The third zone has a smooth inner
surface. Running along the ventral side of the posterior half of the gland is a
prominent groove which merges behind with the lumen of the caudal oviduct
(Text-Fig. 1 b). In addition, there is a less prominent groove on the dorsal
side. Apparently the secretions flow along these grooves, and the lateral
ridges of the fully formed egg capsules are formed by these grooves in the

caudal pole of the nidamental organ, where the egg capsule is moulded into its characteristic shape. Owing to the enormous development of the glands the external muscular couch of the nidamental organ has almost disappeared. The two sides are pressed against each other by their inner surfaces, so that the lumen in a median sagittal section appears as a long narrow antero-posterior slit. Yet the organ is so extensile, that the comparatively large egg capsule from the uterus could easily be pushed up into it. The entrance of the cranial oviduct into the gland is very narrow with the result that the egg, which is big, has to be forced inside the nidamental organ, probably by the peristaltic contractions of the muscles of the oviducal walls.

Histology of the Nidamental Gland

(*a*) *The albumen glands.*—A longitudinal section involving the cranial and caudal parts gives a clear idea of the general structure and arrangement of the gland tubes within the nidamental organ (Text-Fig. 2). Just below
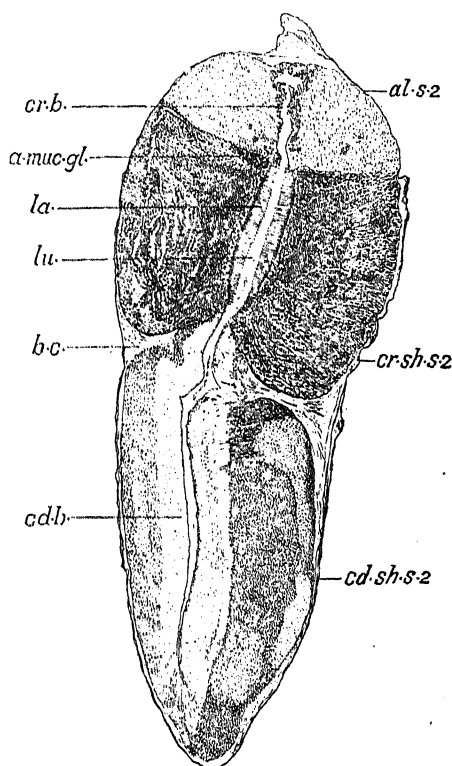
TEXT-FIG. 2

the anteriormost region nearly 1 cm. in width, is the albumen secreting zone. The gland tubes here, are simple and arrange themselves

almost in parallel series. They start blindly at the outer wall, run at first in cranial caudal direction, then get bent and open into the lumen of the organ, in the region of the cranial transverse bands already mentioned. These bands are more or less in the form of low ridges. The gland tubes get cut both horizontally and transversely because of their winding course, transverse sections predominating near the lumen just where the gland tubes get bent when they are about to empty themselves.

The tubules are formed of two kinds of cells, (1) Secretory cells with sparse cilia; (2) Non-secreting cells with prominent cilia and hence called ciliated cells. The secretory cells possess cilia which are not very prominent. The cilia of the ciliated cells are very slender and long, and they aid in wafting the secretory products from the lumen of the tubules into the main central lumen of the nidamental organ. It is doubtful whether the ciliated cells take any part in secretory activity. They have not been observed to contain secretory products. The cytoplasm of the cells forming the albumen glands is alveolar and plasma cells occur in the albuminous region in large nests. They are found in the loose connective tissue between the glands and occasional masses of these lie in contact with large blood vessels. The nuclei of the two sets of cells are at different levels forming two complete concentric rings, the outer ring being formed by the nuclei of the gland cells and the inner by those of the ciliated cells. Roughly speaking an albumen secreting tubule in transverse section (Text-Fig. 3a) is oval in outline, consisting of
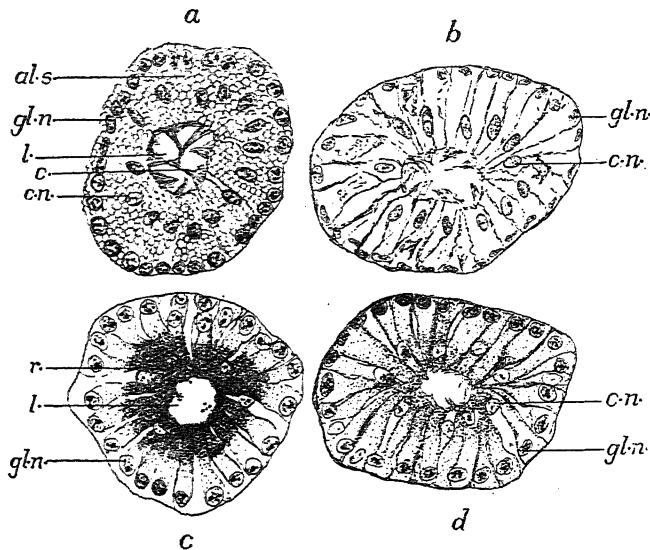
TEXT-FIG. 3

15–20 gland cells, and 7–9 ciliated cells. The nuclei of the former are peripheral and roughly circular. They possess a dark nucleolar body and chromatin granules. It was noticed that though the egg case had already been formed round the albumen covered egg, the cytoplasm of the albumen gland cells was filled with transparent albumen globules packed together so as to form a sort of network. The cytoplasm of the ciliated cells forms a thin rind and is hardly visible as the cells are pressed in between the gland cells. The nuclei of the ciliated cells do not stain as deep as those of the gland cells and are poorer in chromatin. The lumen of the gland tubes are circular and cilia of the ciliated cells reach almost the middle of the lumen. The albumen cells as a whole have clear unbroken margins. When heavy secretion occurs the apical cell walls get ruptured.

The cranial transverse bands bordering the lumen into which the individual glands open are very low when compared with the lamellæ of the shell secreting zone and are nearly as broad as long and quadrilateral in transverse section (Text-Fig. 4). The stroma is made up of vascularised connective tissue containing muscular strands. There are about 20–30 of these bands.
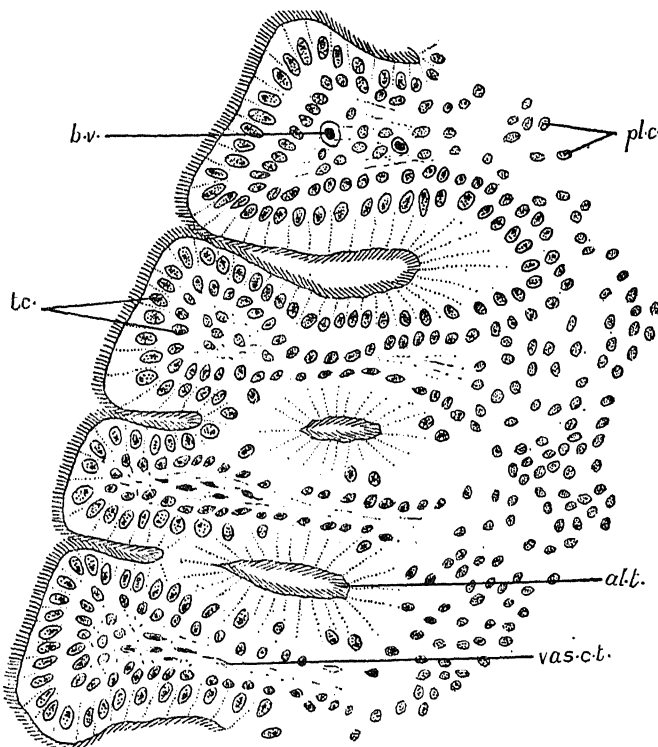
TEXT-FIG. 4

Externally these bands are covered by a two-layered epithelium showing
clearly the respective rows of nuclei one below the other.   These cell layers
with their nuclei correspond to the two layers of cells of the gland tubules,
viz., gland cells and ciliated cells.   The lumen is ciliated in the region of the
cranial transverse bands as also throughout the length of the nidamental
gland.

Mallory's triple stain colours the albumen glands pale blue.   Iron
hæmetoxylin stains them pale grey, van Gieson pale pink.   This latter
counterstain brings out the cilia well.   In the case of Thionin blue, certain
regions stain blue while others appear purple.   The purple colouration is
prominent only when stain is fresh, and is washed away as sections
are graded up in alcohols.   The curious phenomenon of part of
the albumen glands staining blue, while others are coloured bright
purple (mucus test) is in accordance with Borcea's statement: "That the
albumen of the Scyllium egg presents more the characters of mucin than
albumen proper".   He says, that in Rays, it is characteristic that the whole
of the first zone of the nidamental gland secretes mucin.   Borcea has also
verified that among Elasmobranchs there are variations in the nature of the
albumen secreted by the first zone of the nidamental gland.   He
compares this with the observations of Tarchanow and of Lataste (1889)
on the albumen of bird's eggs.   Tarchanow observes that the eggs of nidi-
fugous birds (where the young ones are hatched in a very advanced state as
for example the egg of fowl) possess the ordinary albumen or white of the
egg.   But among nidiculous birds where the young are hatched (precocious),
or incompletely developed (pigeons, sparrows and crows), the egg possesses
a particular albumen, which has mucin characteristics and remains trans-
parent after coagulation by heat.   This albumen is alkaline to litmus and is
apt to imbibe and swell out in water.   He called it Tatalbumen or "Tata-
blanc".   The two kinds of albumen are generally linked because Tatablanc
easily transforms itself into the white of ordinary eggs under the influence of
the yellow.   Lataste shows that this particular albumen exists also in notable
proportion in the white of the egg of hen, and it seems no other than mucin.
This is also in accordance with Borcea's statement (1904) that in the
Elasmobranchs there are variations in the nature of the albumen secreted by
the first zone of the nidamental organ.   In *Acanthias* and Rays, Borcea says,
the white of the egg is formed exclusively of mucin; among others by
albumen proper and mucin.   Probably, the facts observed in *Chiloscyllium
griseum* indicate, that the white of the egg is formed either of albumen proper
and mucin, or following Tarchanow, it is composed of Tatablanc which has
mucin characteristics.

(b) *Histology of the anterior mucous glands.*—Just below the region of the albumen glands and lying in between the former and the shell gland region is a horizontal row of transversely cut tubules which in the entire gland will naturally be in the form of a partition between the albumen and shell gland zones (Pl. V, Fig. 2). These glands are easily identified to be mucous or slime secreting glands, due to their dark purple reaction with thionin. The gland cells provide a thin layer of mucous secretion over the already albumen covered egg to prevent friction between the albumen and forthcoming shell secretion. Mallory's stain colour these glands pale blue, and other stains leave them colourless. They have almost the same structure as the albumen glands, with gland cells and ciliated cells, but are slightly larger in size. The basal nuclei are shrunken and poor in chromatin (Text-Fig. 3b) The slime glands open into the lumen between the cranial bands and the lamellar region.

(c) *Histology of the shell glands.*—The shell secreting region is the third zone in the nidamental gland. These glandular tubes are also larger in size than the albumen glands. They commence blindly at the periphery and run towards the lamellar zone so that in a sagittal section they are all cut horizontally more or less. They show considerable similarity in structure with the albumen glands. In the active state they are closely packed together with hypertrophied capillaries, and a greatly reduced connective tissue stroma between them. The boundaries of the cells can only be recognized with great difficulty. Where heavy secretion occurs, the apical cell-walls are ruptured, whereas in resting condition the cells have clear unbroken margins. The shell secreting tubules are differentiated into two sets; a cranial set of tubules and a caudal set of tubules. Just below the horizontal row of slime glands is a comparatively narrow zone of transversely cut tubules in 5–7 rows which stain jet-black (Pl. V, Fig. 2), while the rest of the shell secreting tubules stain greyish black. With van Gieson counterstain the former remain black while the other shell tubules take a straw-yellow colour.

The cytoplasm of these shell tubules is filled with dark staining granular secretions; but in some, towards the lumen of the transversely cut tubules the secretion is very dense, and forms a jet-black ring (Text-Fig. 3c), bordering the circular lumen of the tubule, so that the inner ring of nuclei of ciliated cells is greatly obscured. The shell tubules are composed of gland cells (20–24) and ciliated cells (7–10) (Text-Fig. 3d). Gland cells are loaded with granular secretion that fill the cell cytoplasm completely causing the cell boundaries to rupture. Probably, once the fish attains maturity during the breeding season, the nidamental glands remain continuously active and the stimulus

of the egg in the cranial oviduct does not seem to be absolutely necessary for the gland to start functioning. For, as soon as the egg cases in the uteri are liberated into the sea, new eggs from the well-developed median ovary may descend into the oviducts; hence the nidamental organs are always kept in a state of activity with little or no interval of rest. It is obvious that this process is rapid, as it is very difficult to obtain specimens with egg inside the cranial oviduct, or nidamental gland. The specimens examined were either in the spent condition or with the egg cases in the uteri. Other workers have encountered the same difficulty of not obtaining even a single specimen with the capsule in the course of formation. Once the egg has reached the oviduct, events seem to move rapidly. It cannot be otherwise, as several pairs of eggs have been known to be laid in aquaria in the course of one or two weeks.

The nuclei of the gland cells of the shell secreting tubules are larger and more circular than those of the albumen glands, while those of the ciliated cells are oval. The nuclei of the shell tubules on the whole are poorer in chromatin contents than the albumen glands. The cilia are very fine and not prominent. The gland tubes open into the lumen of the nidamental gland in the lamellated zone.

(d) *The lamellæ, their arrangement and structure.*—The internal folds of the mucosa in the shell secreting region are in the form of lamellæ covered by a strong layer of connective tissue, which lies deeply bordering the glandular region. This connective tissue is produced into the corium of the lamellæ which is vascular. There are about 75 lamellæ, all horizontal, the anteriormost ones of medium size, with flattened tips. In the middle they are very long and typically differentiated with curved ends (Pl. V, Fig. 3). Posteriorly they again shorten in length. Occasionally the lamellæ are dichotomous. They are very narrow at the base where the epithelium of the two sides is almost in contact. Towards the middle they broaden out in section and are filled with connective tissue with vascular dilations containing large blood corpuscles. Towards the ends, they project freely into the lumen of the gland. The outer lining epithelium is uniformly ciliated. The epithelial cells are less high at base of the lamellæ, where they are cubical with rounded nuclei. In the dilated part of the lamellæ the epithelium is composed of cylindrical cells with elongated nuclei. Each lamella is accompanied by two smaller linear processes (like the linear stipules of a leaf) one on each side. The epithelium of these is composed of cubical cells with rounded nuclei similar to those of the bases of lamellæ and is continuous with the epithelium of the latter. These processes Widakowich calls "Zottchen", meaning tufts. The lamellæ project in regular series without interruption while occasional irregularity is found in the arrangement of the tufts. Sometimes, a lamella has only

one tuft. But wherever gland tubes open into the lumen they empty themselves between the two tufts of two adjacent lamellæ. Thus the glandular opening is guarded by the two tufts accompanied by the two respective lamellæ on either side. The cilia lining the lumen of the gland tubes are very prominent here, and matted. Where the glands open, the secretion is in the form of thick granular filaments staining yellowish-brown with van Gieson counterstain after Iron hæmetoxylin similar to that found in the gland cytoplasm. It would be interesting to know whether these are the remains of the secretion that went to form the egg capsule, or can it be that the nidamental gland is in a fresh state of activity for a forthcoming egg ?

From the nature of the lumen bordered with lamellæ, there can be no doubt that Widakowich is correct in his supposition that the lamellæ and tufts have the power to roll the plastic secretion into a layer of suitable thickness, thereby, the egg getting surrounded with as many layers of secretion as there are interlamellar spaces. Through the pressure of the glands opening at their bottom the tufts give a direction to the lamellæ, in the rolling of the layers. In many sections the ciliated epithelium bordering the lamellæ seemed rubbed off, or broken up into bits. Hence the epithelium was not entire. This occurred especially at the tips of the lamellæ and the vascular core projected bare. This is a characteristic feature of the epithelium, due to its being constantly renewed, and not due to faulty razor or careless sectioning, because wherever the lamellæ were bare, several bits of the epithelium were found thrown into the lumen. It was also noticed that new epithelium was rapidly being formed. Thus the ciliated epithelium of the lamellæ is in a constant state of renovation.

(*e*) *Histology of the posterior half of the shell secreting zone.*—The *caudal zone* of the nidamental organ is densely packed with narrow shell secreting tubes. This region is separated from the anterior shell glands, or the zone of lamellæ on both sides of the lumen, by a broad band of parenchymatous tissue which is very vascular (Pl. V, Fig. 4). This parenchymatous band in the nidamental gland of *Chiloscyllium* is absent in that of *Scyllium canicula* described by Widakowich. In the latter, there is no distinct division of the nidamental organ into caudal and cranial portions. There are slight differences even in the shape of the nidamental glands in the two cases.

The *caudal zone* (Text-Fig. 2) starting just below the lamellated region, in sagittal section, presents the following structure. On either side of the continuous slit-like lumen of the nidamental organ lie an outer broad longitudinal band of shell secreting tubules, and an inner narrower band of transversely cut mucous tubules. The latter have clear transparent cytoplasm,

and are situated on either side of the lumen just outside the region of the caudal bands. On close examination one finds that these are merely the inner extremities of the caudal shell tubules, which assume mucous secreting function, when necessity for mucin occurs. This view is supported by the fact that caudal shell tubules and caudal mucous tubules are simply different parts of the same tubule in transverse section. Only the staining reactions vary, due to the difference in the nature of secretion. Moreover, in certain longitudinal sections of tubules (Pl. V, Fig. 5), the cytoplasm is loaded with granular secretions at their broader outer ends, while at their inner extremities the cytoplasm is devoid of secretions and is transparent (Pl. V, Fig. 6; P. VI, Fig. 1). Thick streaks of granular secretion occur in the lumen. The secretions stain straw-yellow with van Gieson and greyish-black in Iron hæmetoxylin. At the outer (granular) end of tubule, the cell outlines are clearer, and both in horizontal as well as transverse sections two sets of nuclei at different levels are obtained. The outer or peripheral nuclei are those of the gland cells and the inner nuclei those of the ciliated cells. At the inner narrower ends of the tubules, however, the peripheral nuclei get shifted towards the lumen and are almost in line with those of the ciliated cells. The nuclei in these regions are shrunken and chromatin contents very poor. It is very probable that these regions represent the washed-out condition of merocrine gland cells. The caudal tubules as a whole are narrower than the cranial set. There is a gradual reduction in size even in the former, for the tubules diminish considerably in diameter from just below the lamellated zone to the posterior-most extremity of the nidamental gland.

The caudal slime glands function last of all, and provide mucin to be poured over the egg case to help it to slide freely into the caudal oviduct, without friction between the two surfaces. The lumen of the nidamental gland in the caudal zone is bounded by low ridges (Text-Fig. 5a), their stroma consisting of connective tissue. Between these the outgoing ducts of the narrow shell glands lie. Their surface is covered uniformly with cilia.
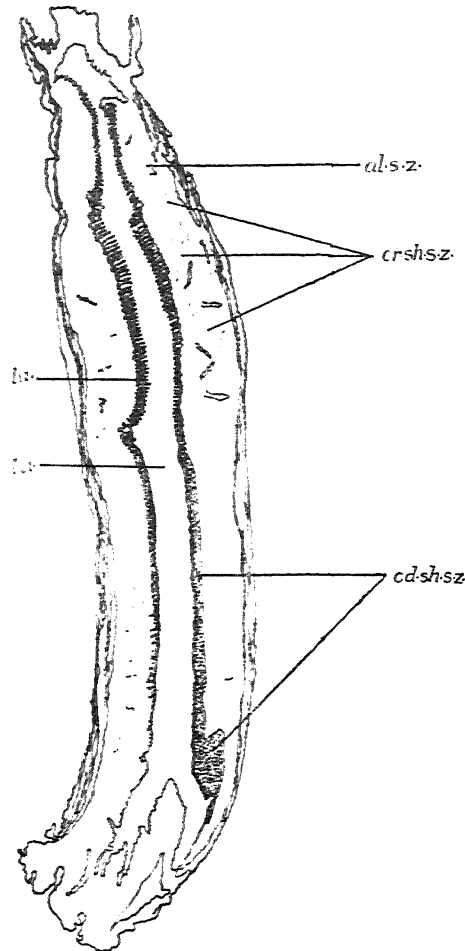
Development of the Nidamental Organ

In the advanced embryos and young animals the gland presents the form of a slight swelling of the oviduct, lined in the interior by a simple epithelium (Text-Fig. 6). The elements of this epithelium proliferate actively and then the surface presents some folds, in the deeper parts of which the glands are formed by budding. The glandular tubes do not acquire the special characters of the adult animal until maturity and once the adult stage is attained the characteristic features develop very rapidly. At first, there is no distinction between secretory or gland cells and ciliated cells. All the cells
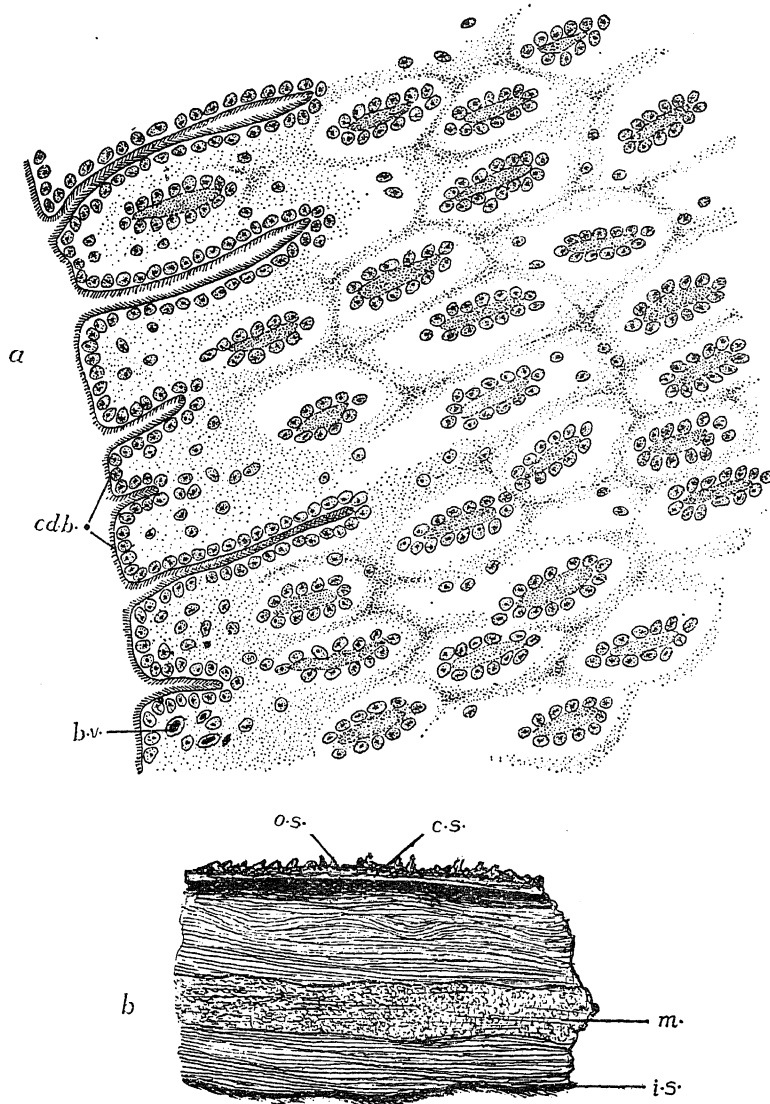
TEXT-FIG. 5

are ciliated and the nuclei at the same level (Pl. VI, Fig. 3). Later, multiplication of cells occurs and certain nuclei get shifted towards the lumen of the gland tubule; thus two concentric rings of nuclei are obtained. Intermediate stages in this shifting could be seen in certain sections. The outer ring consists of nuclei of the gland cells which are secretory in function, while the inner are the nuclei of the ciliated cells proper, which appear pressed between

two secretory cells. This is also in accordance with Borcea's observations
(1904). It is very interesting to note that the caudal shell tubules are
the first to develop (Text-Fig. 6). Shell (serous) secretions take a longer
time to mature than the other secretions, hence it is necessary that the
cycle in the shell tubules should start earlier. The lamellæ develop typically
in the anterior shell secreting zone quite early even before the gland tubes
could develop in that region (Text-Fig. 6).

TEXT-FIG. 6

Nidamental Organs of Chiloscyllium griseum *compared with those of*
Scyllium canicula *and a Few Madras (Viviparous) Elasmobranchs*

A brief account of the nidamental glands of other Elasmobranchs
examined may not be out of place.

The nidamental glands of *Chiloscyllium griseum* bear great resemblance to those of *Scyllium canicula* described by Widakowich, as well as to those of *Scyllium catulus* worked out by Borcea in 1904. The resemblances are striking and only to be expected in such closely related oviparous Elasmobranchs. The egg as it passes the nidamental gland is covered first by the albuminous secretion from the anterior region of the nidamental gland, then by the horny secretion which hardens to form the capsule. This egg capsule is liberated by the fish into the sea and there the egg develops into the embryo which on completion of its development escapes from the capsule (Gopala Aiyar and Nalini, 1938). It is well known that the uterus is better developed in the viviparous forms than in the oviparous as here the embryos are lodged and nourished for a considerably long time within the uterus of the mother. In oviparous forms, where the embryos are not nourished by
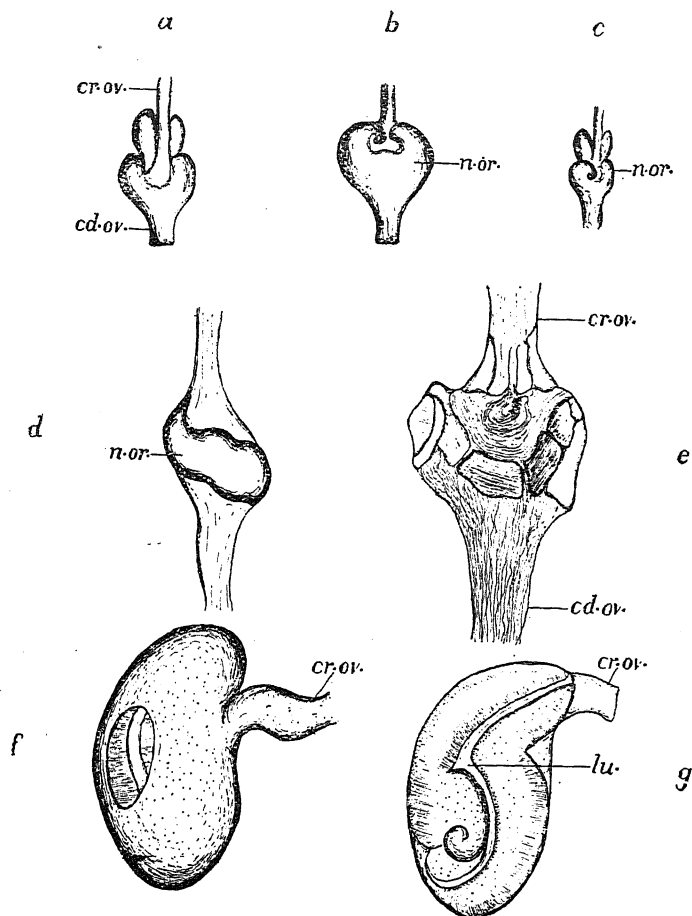
TEXT-FIG. 7

uterine fluids, but the uterus only serves to harbour the egg (lodged in a capsule) for a short time, the structure of the uterus is simplified to a certain extent, though muscularity has to be retained for the purpose of expelling the eggs. The nidamental glands on the contrary are better developed here. Examination of several mature viviparous Elasmobranchs through the courtesy of Miss Mahadevan, has shown that the glands are present in viviparous forms as well. In fact, most, if not all the different viviparous Elasmobranchs examined in the course of my work, had some indication at least of the nidamental glands. *Narcine timlei* and *Astrape dipterygii* were the only two forms that lacked in these organs completely. In *Scoliodon walbheemi* (Text-Fig. 7a), *Sc. dussumieri* (Text-Fig. 7b) and *Sc. palasorrah* (Text-Fig. 7 c) the nidamental glands are quite big in comparison with size of fish and in the mature forms, about 1″ long and ½″ broad. They are heart-shaped with two anterior horns and consist of two sets of glands. When the uteri of these specimens were slit open carefully the embryos were found to be covered by a very thin transparent shell membrane in all cases, and remnants of the shell membranes were found to be retained by the uteri of fish where the embryos had already been liberated. In one specimen of *Pristis cuspidatus* each uterus was distended with a complete bag-like structure composed of softly delicate and smooth shell membrane, within which large yolky masses were lodged wrapped in gelatinous albumen. One egg case contained three eggs, the other one, *Pristis* being viviparous the egg case is merely to afford temporary protection for the eggs within the uterus and hence it is delicate in texture. The nidamental glands in *Pristis* are large and cup-like with a very spacious cavity (Pl. VI, Fig. 6). Here the walls are not as thick and softly glandular as in *Chiloscyllium*, but all the same, are composed of gland tissue. The inner surface is seen to be differentiated into three zones, the anterior two consisting of lamellæ. Sections of a strip show that two sets of glandular tubes, albumen and shell secreting respectively, compose the wall of the nidamental organ, though the secretion in the shell tubules was not very profuse.

In *Rhinobatus granulatus*, another ovo-viviparous Elasmobranch, the nidamental glands in the mature fish are huge, kidney-shaped, solid bodies (Text-Fig. 7 f), composed of closely packed filamentous tubules. Thus we find that even in the viviparous Elasmobranchs, the nidamental glands do occur, and are not wholly functionless. These examples lend sufficient proof to the fact that even in the viviparous forms the embryos are for sometime covered by a thin shell membrane secreted by the nidamental organ and which gets ruptured in the uterus itself before the embryos are liberated. For this purpose the nidamental organs are retained in the viviparous forms

though they are present in a comparatively reduced condition. In both oviparous and viviparous forms, in the immature stages, however, the glands are inconspicuous and hardly distinguishable from the oviduct. They are represented by a very slight dilation of the walls of the oviduct and it is only with approaching maturity that these walls become glandular and functional.

Structure of the Egg Case

The egg case of *Chiloscyllium* is in the form of a long pod, slightly a-symmetrical and made up of two lateral halves (Gopala Aiyar and Nalini, 1938). It more or less takes the mould of the caudal pole of nidamental organ from the level of the third zone. The wall of the capsule is composed of several layers. Each layer is formed of many tiny filaments lying very close together. In a transverse section of the shell these filaments give rise to a beaded appearance.

The egg covered with albumen, as it descends through the lamellated zone of the shell tubules, gets enveloped by thin streaks of secretion, poured from between the lamellæ. The secretions are formed in the form of granules in the cytoplasm, then the granules by some means arrange themselves in the cells in the form of delicate strings and these flow out (Pl. VI, Fig. 4). The flow is continuous and results in the formation of the layers. Each layer is made up of as many filaments as there are inter-lamellar spaces, but the filaments of each layer almost immediately run into each other forming a complete sheet. The stickiness of the secretions renders the fusion of the sheets perfectly easy to form the horny egg capsule. The final secretions are drawn out into the anchoring filaments when the egg is actually passing into the uterus (Pl. VI, Fig. 5), so that the silky twisted filaments of the ventral edge as well as the smaller filaments fringing the margin, are all in continuity with the outermost longitudinal striations of the egg case. These filaments could be traced, running longitudinally along the surface of the capsule for some distance, clearly indicating that they are in continuity with the outermost striations of the capsular wall.

A critical study of the cross-section of the capsular wall shows three distinct layers (Text-Fig. 5b). In Mallory's triple stain the two outer zones stain bright red, a characteristic test for keratin, and these zones are composed of horizontal beaded striations, each striation representing a layer of secretion. The middle zone stains blue and also consists of beaded striations.

The red zone representing the free surface of the capsule is composed of two regions, the outermost being the darkest, and separated by a very narrow streak of clear space from the adjacent layer, which may represent a slight pause in secretory activity, the two parts being formed at different levels of

the gland as the egg slips down the lumen. The outermost surface also consists of tiny protuberances. This may represent the cut ends of the outer longitudinal striation, or may be due to the mould of the caudal oviduct into which the capsule descends in a soft condition.

Mode of Formation of the Egg Case

There is a good bit of uncertainty about the exact stage at which the horny case is secreted around the fertilized Elasmobranch egg. Most textbooks just say that the shell is secreted while the egg is passing through the nidamental organ, or pass over the matter in silence. Of late years the question seems to have received very little attention and within the last twenty years, Hobson is probably the only writer who has contributed something towards this problem.

Gerbe (1872) and Perravex (1884) agree that the albumen and shell are secreted simultaneously around the egg. Perravex, however, concluded that although secretion of shell starts before egg reaches the gland, the shell is actually formed round the egg. In 1890, Beard stated that in skates, it is almost certain that the lower half of the purse is formed before the egg arrives at the oviducal gland. He also suggested that the closure of the purse is effected only after the arrival of the egg, which is in accordance with Hobson's view. Borcea (1904) believes that normally the ovum incites the secretion of the oviducal gland and adds that other stimuli may have the same effect. He did not apparently come across specimens directly confirming his view although he cited the occurrence of fully formed egg cases containing only albumen without the egg, as a proof that the egg is not the only stimulus which can cause secretion of shell. Sedgwick (1905) is of opinion " that the shell is formed round the ovum, and its albumen in the lower dilated part of the oviduct, but the material of which it is composed is secreted by the oviducal or nidamental gland ". Widakowich (1906) gives a detailed account of the formation of the egg case in *Scyllium canicula*. He has observed that often two eggs leave the median ovary, and when they reach the cranial oviduct they are pushed by the peristalitic contractions of the oviducal walls, into the nidamental gland, where the secretions are poured over them. The plasticity of the secretion unites the layers firmly. The anterior and posterior ends of the shell in *Scyllium* are continued into filaments. The caudal processes of the egg case, he recognized as being laid down during the passage of the egg down the cranial oviduct. The body of the case however, he thought, was formed during subsequent movement of the egg through the gland. For the longitudinal striations of the shell on the outside, Widakowich finds it difficult to give an explanation.

Dean (1906) describes the formation of the egg case in Chimæroid fishes. The folds and ridges of the oviduct in the posterior region of the capsular gland (nidamental organ), he says, models the secretion of the gland into the beginning of the capsule, and the oviduct just below the capsular gland forms an exact mould for the capsule. As the egg descends the other parts such as tail sheath and capsular filaments are added on gradually.

Hobson's recent statement of 1930, in his note on the formation of the egg case in the skate *Raia radiata* is however, quite different. Out of the 150 or more gravid females he examined, one luckily, according to him had the egg case in the process of formation. Each oviduct was swollen with a mature egg devoid of albumen or shell, anterior to the nidamental organ. Behind the latter, on either side, the oviduct was greatly distended by the posterior half of the capsule which was already formed. This was, however, devoid of albumen and was quite empty. The dorsal and ventral walls of this half-formed egg case were well separated from one another, so that there was an opening at its anterior end to receive the egg. Thus at least half and possibly considerably more of the egg case, Hobson states, is formed in advance, long before the egg comes in contact with the nidamental organ. This belief of Hobson's, in part formation of the egg capsule is quite contrary to that of Widakowich. Hobson records a single case only. If the phenomenon were normal it should have been met with most frequently, for he states he examined several specimens. Further, after the egg cases were fully formed, there should have been definite traces of a transverse suture between the two halves, denoting the line of fusion. He does not record any such suture in the numerous fully formed egg cases seen by him. Another difficulty in Hobson's statement, is how, remembering the highly sticky nature of the secretions, the dorsal and ventral walls of the half-formed egg case could remain well separated from one another, even though there was no egg in between, to keep them apart. The secretion being so very plastic, it is difficult to conceive how the front ends could remain open. Unless there was something in between, the two sides would naturally come together and stick.

Prof. R. Gopala Aiyar has just drawn my attention to Metten's paper (1939) on the reproduction of the dog fish *Scylliorhynus canicula* and I have now re-examined my sections and photographs taken in 1938 when my paper (Nalini, 1938) was read before the Jubilee Session of the Indian Science Congress. Spiral clusters (Pl. VI, Fig. 4) had been noticed even then but were considered as commencements of the shell secretion in the form of very slender spiral threads. It is quite possible that shell filaments and sperms occur in the lumen inextricably mixed up. The question whether sperms are actually

present inside the nidamental organ is being thoroughly investigated in *Chiloscyllium griseum*.

As regards the formation of the egg case Metten concludes from his observations that the presence of the ovum inside the nidamental gland is not necessary for the commencement of the secretions. He further states that the egg case is partially formed before the arrival of the ovum. Fig. 7 in Pl. VI shows the egg case in the fully formed condition inside the nidamental gland of *Chiloscyllium griseum*. Several gravid females recently obtained from Calicut showed the same condition. While this fact alone cannot be adduced as evidence for the statement that in *Chiloscyllium griseum* the egg case is not formed in parts, still it is highly suggestive of it, if taken in conjunction with the fact that an examination of the fully formed shell does not give any indication of the formation of the egg case in parts by the presence of a suture or line or any difference in consistency between the various parts of the shell substance.

In the case of *Chiloscyllium* however, the shape and structure of the egg capsule being quite different, Hobson's views also are not applicable. There is not the least indication of an anterior lid here, but the whole capsule is in the form of a long pod, of entire continuous edges and thickened rims. Though it is difficult to say at exactly what point the capsule is moulded I am inclined to agree with Widakowich and Sedgwick that the secretions are poured over the egg after it reaches the nidamental organ. Probably the mode of formation of the egg case varies in the different species of oviparous Elasmobranchs. Further work is needed before the question can be answered satisfactorily.

Summary

1. *Chiloscyllium griseum* is a typically oviparous Indian Elasmobranch. The nidamental glands which secrete the egg albumen and the egg case are very prominent and of maximum activity. The lumen of the gland is continuous with that of the cranial and caudal parts of oviduct.

2. The gland consists mainly of three kinds of tubules, *viz.*, albumen, mucous and shell secreting. They are all simple with circular lumen and more or less of the same structure. They empty into the lumen of the nidamental organ at different regions. The albumen glands comprise the anteriormost or first region of the nidamental organ. Next is the partition formed of large mucous glands. The shell secreting region forms the major portion of the organ. This zone is divided into two, consisting of a cranial and a caudal set of tubules respectively. Small mucous glands slightly different from the large cranial mucous tubules occur in large numbers in the caudal half of the nidamental organ.

3.   All the gland tubes irrespective of their function consist of two types of cells, gland cells and ciliated cells.   The former are greater in number and are secretory in function and possess large peripheral nuclei.   The ciliated cells occur lodged in between gland cells and have slightly elongated central nuclei surrounded by clear cytoplasm.

4.   The method of secretion inside the gland tubes and the laying down of the albumen and shell layers are dealt with in detail.

5.   A brief comparison of the nidamental organs of *Chiloscyllium griseum* with those of the oviparous European *Scyllium canicula* as well as a few (viviparous) Elasmobranchs of Madras is instituted.

6.   Various views in regard to the method of capsule formation are discussed.

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EXPLANATION OF TEXT-FIGURES

TEXT-FIG. 1. (*a*) Nidamental gland slit from the dorsal side.
             (*b*) Nidamental gland slit open to show the ventral groove.

  ,,   2. Median sagittal section of entire gland.

  ,,   3. Transverse sections of the different gland tubules.
             (*a*) Albumen tubule.
             (*b*) Large mucous tubule.
             (*c*) & (*d*) Shell tubules.

  ,,   4. Cranial bands of albumen secreting region showing the outer epithelium con-
             sisting of two cell-layers.

  ,,   5. (*a*) Shell secreting region, caudal zone.
             (*b*) Transverse section of shell case.

  ,,   6. Sagittal section of an undeveloped nidamental organ.

  ,,   7. Nidamental glands of a few other Elasmobranchs.
             (*a*) *Scoliodon wqlbeehmi.*
             (*b*) *Scoliodon dussumieri.*
             (*c*) *Scoliodon palassorah.*
             (*d*) Young nidamental gland of *Rhinobatus granulatus.*
             (*e*) The same slit open.
             (*f*) The fully developed gland, a portion of the inside exposed.
             (*g*) The same with inside fully exposed showing the lumen.

EXPLANATION OF PHOTOGRAPHS

PLATE V

FIG. 1. Photograph of reproductive system of *Chiloscyllium griseum*.

„ 2. Photomicrograph of section passing through partition formed of large mucous glands between the albumen and cranial shell tubules. × 80.

„ 3. Photomicrograph of section passing through the cranial shell secreting region showing the lamellæ and tufts at the bases of which the shell tubules open into the cavity of the nidamental organ. × 80.

„ 4. Photomicrograph of section through the non-glandular partition between the cranial shell secreting and the caudal shell secreting portions. × 80.

„ 5. Photomicrograph of caudal shell tubules. Note the active condition of the tubules, in one half of the photograph and the resting condition in the other. × 80.

„ 6. Photomicrograph of the active condition of the shell tubules with secretion in the cell cytoplasm. × 400.

PLATE VI

FIG. 1. Photomicrograph of the resting condition of the shell tubules with the secretion actually in the lumen in the form of a black streak. × 400.

„ 2. Photomicrograph of a portion of an undeveloped nidamental gland. × 80.

„ 3. Photomicrograph of a portion of the same to show clearly the structure of the very young gland tubules. Note fewness of number of the cells and the presence of a single layer only. × 1800.

„ 4. Photomicrograph of a portion of a cranial shell tubule showing the cells heavily loaded with granular secretion and the lumen with several delicate shell filaments just forming. × 1800.

„ 5. Photomicrograph of the caudal shell secreting region at the caudal bands showing two filaments of secretion flowing out from the lumen of the shell tubules into the cavity of the nidamental organ. × 80.

„ 6. Photograph of nidamental gland of *Pristis cuspidatus*.

„ 7. Photograph of a fully formed egg case of *Chiloscyllium griseum* inside the nidamental gland.

LIST OF ABBREVIATIONS

act. cond.	..	..	Active condition of shell tubules.
al.s.	..	..	Albumen secretions.
al.s.z.	..	..	Albumen secreting zone.
al.t.	..	..	Albumen secreting tubule.
a.muc.gl.	..	..	Anterior mucous glands forming the partition.
b.c.	..	..	Band of connective tissue between cranial and caudal halves of the nidamental organ.
b.v.	..	..	Blood vessel.
c.	..	..	Cilia.
cd.b.	..	..	Caudal band.
cd.ov.	..	..	Caudal oviduct.
cd.sh.s.z.	..	..	Caudal shell secreting zone.

cl.	..	.. Cloaca.
c.n.	..	.. Nucleus of ciliated cell.
cr.b.	..	.. Cranial band.
cr.ov.	..	.. Cranial oviduct.
cr.sh.s.z.	..	.. Cranial shell secreting zone.
c.s.	..	.. Clear space in section of shell showing pause in secretory activity.
d.gr.	..	.. Dorsal groove.
d.l.		.. Dichotomous lamella.
fil.s.	..	.. Filamentous secretion in the lumen of shell tubule.
gl.n.	..	.. Nucleus of gland cell.
gl.o.	..	.. Gland tubule (shell secreting) emptying into the lumen of nidamental organ between two tufts.
gr.s.	..	.. Granular shell secretion in cell cytoplasm.
i.s.	..	.. Inner surface of shell (egg capsule).
l.	..	.. Lumen of gland tubule.
l.a.	..	.. Lamella.
lu.	..	.. Lumen or cavity of nidamental organ.
m.	..	.. Middle zone of shell.
n.or.	..	.. Nidamental organ.
od.o.	..	.. Oviducal opening.
o.s.	..	.. Outer surface of shell with protuberances.
pl.c.	..	.. Plasma cells.
r.	..	.. Ring of dense black secretions in cranial shell tubule.
r.cond.	..	.. Resting condition of shell tubules.
t.	..	.. Tuft.
te.	..	.. Two-layered epithelium at the lumen of nidamental organ.
ut.	..	.. Uterus.
vas.c.t.	..	.. Vascular connective tissue.
v.gr.	..	.. Ventral groove.

od. o.

cr. ov.

n. or.

cd. ov.

ut.

cl.

1

al. s. z.

cr. b.

a. muc. gl.

cr. sh. s. z.
t.

vas. c.t.
d. l.

2

t.

la.

vas. c. t.

gl. o.

3

cr. sh. s. z.

b. c.

cd. sh. s. z.

4

r. cond.

act. cond.

5

gr. s.

c. n.

gl. n.
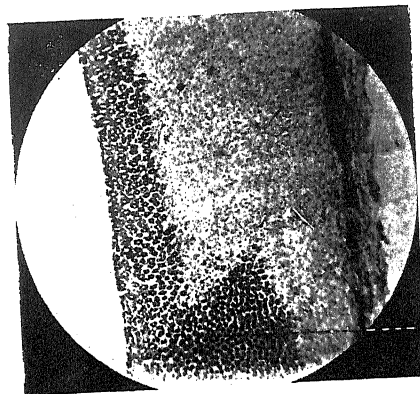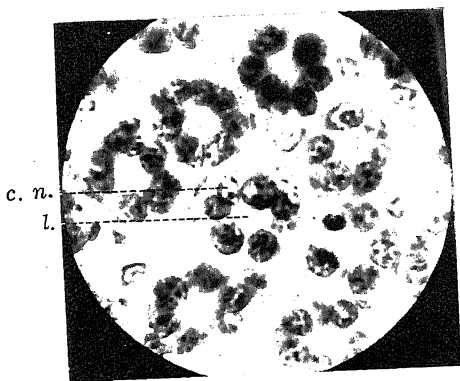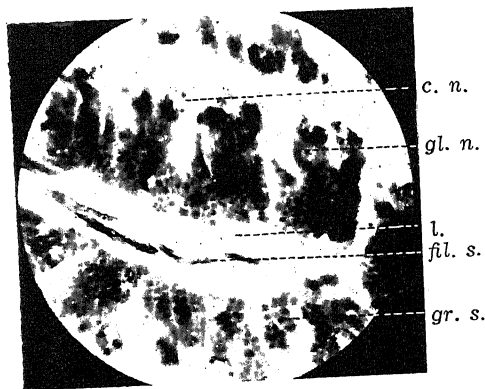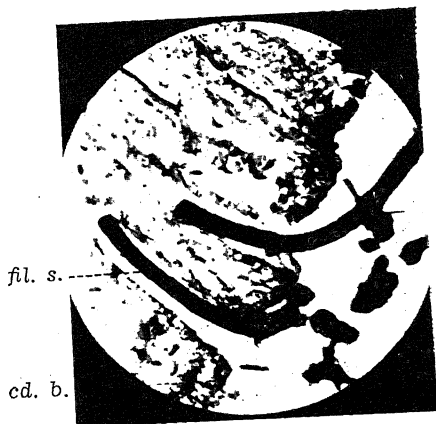
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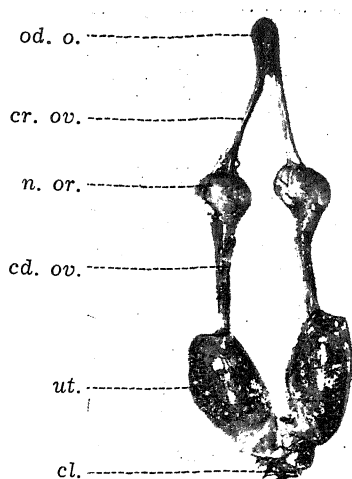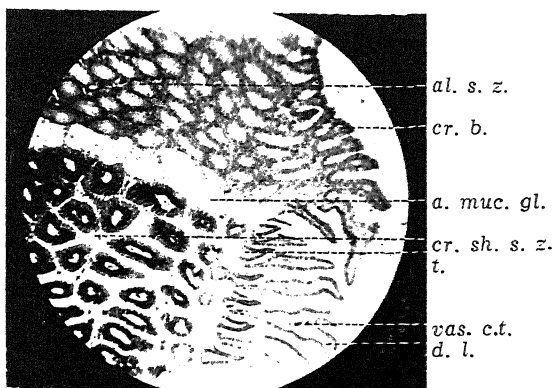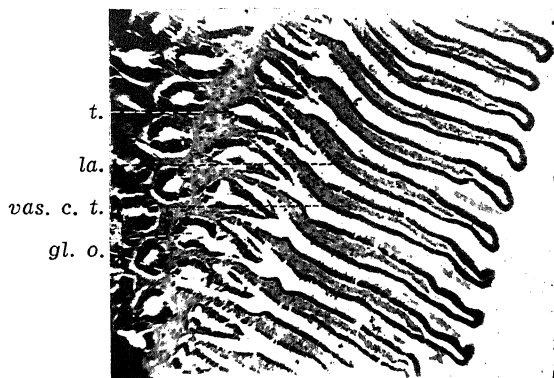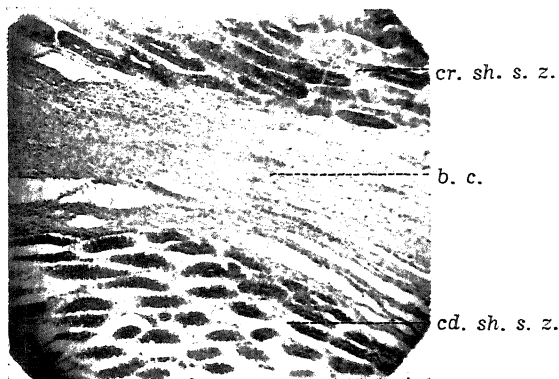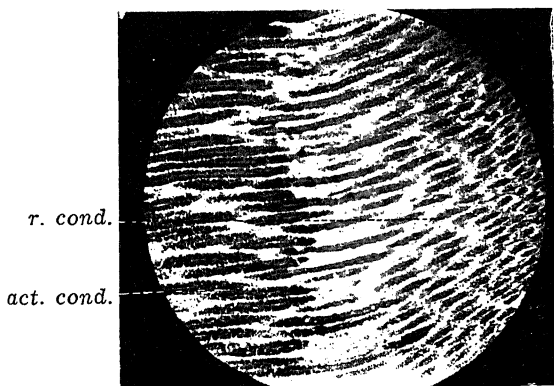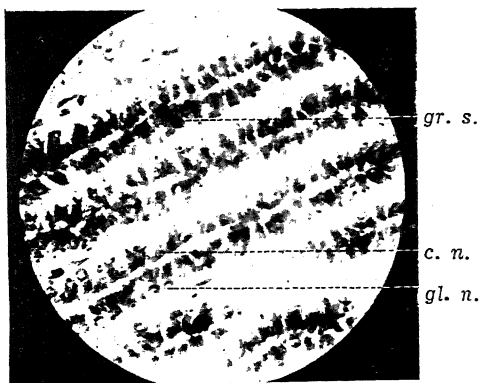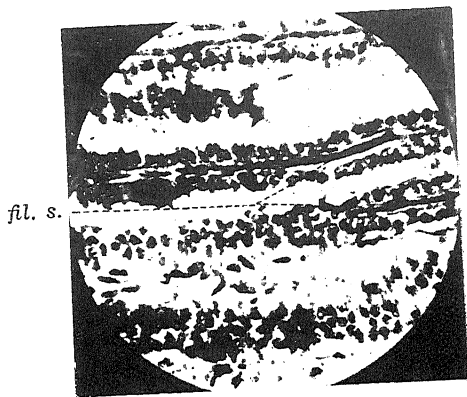
ROYITE, A NEW VARIETY OF QUARTZ, FROM THE JHARIA COAL-FIELD

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Introduction

THE mineral occurs in more or less reticulated or radiating blades (Fig. 1), deposited along the joint planes of the laminated sandstones and shales of the eastern portion of the Jharia Coal-field. It was first discovered by the author along the joint plane of a bed of ferruginous shaly sandstone, behind Rao Bahadur D. D. Thacker's Labourers' School at Pure Jharia Colliery. Subsequently, a specimen of the same mineral was collected by the author from the debris of the IX seam incline of Messrs. Dana and Premji in Tisra area. Here the mineral occurs along the joint plane of a carbonaceous and micaceous shale. Later on, Mr. G. C. Chattopadhyay found a specimen of this mineral in the debris from the shaft of Ghansadih Colliery in Godhur area. The mineral at this locality is deposited along the joint plane of carbonaceous shale. Recently, the author (with his geology students) has noticed the same mineral in the debris from the incline of VI and VII seam, Basaria Colliery, on the western bank of the Bansjor stream. At this locality, the mineral occurs as beautiful radiating blades along the joint planes of carbonaceous or micaceous shales and ferruginous sandstone. Thus the mineral has been found all over the eastern portion of the Jharia Coal-field, though always in very limited quantity.

Chemically, the mineral is composed mostly of silica. Physically, however, it is so unlike ordinary quartz that it is difficult to identify it correctly, unless one knows its chemical composition. It has been, therefore, thought advisable to give a new name to this variety.

Prof. S. K. Roy, during his last visit to Zurich, showed the Tisra area specimen of the mineral to Prof. P. Niggli who also agreed that it was a new mineral; but at that time, the chemical analysis of the mineral was not available.

Physical Characters

The mineral is brownish-black or black in colour with no streak. In thin fragments it usually shows greyish colour. The mineral occurs in long

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but very thin prismatic crystals, varying from $\frac{1}{2}$ inch to $1\frac{1}{2}$ inches in length, $\frac{1}{8}$ inch to $\frac{1}{4}$ inch in breadth, and $\frac{1}{30}$ inch in thickness. The mineral blades from Basaria Colliery are, however, thicker than those from other localities, and vary from $\frac{1}{8}$ inch to $\frac{1}{16}$ inch in thickness. The crystals are sometimes tapering at one end. The hardness of the mineral is 7, and its specific gravity is 2·65.

The mineral shows good prismatic cleavage. It has vitreous luster, shining on cleavage faces, due to which the extremely thin black blades of the mineral, when deposited along the joint planes of black carbonaceous shale, can be easily distinguished from the latter. The Basaria specimens, however, show on cleavage surfaces a characteristic schiller lustre. The prismatic crystals of the mineral show transverse cleavage cracks, which make the crystals break in rhomb-like form. The Basaria crystals, due to their being thicker than those from other localities, break in small six- or four-sided prismatic columns. None of the cleavage faces of these cloumns gave ideal shining surface for goniometric measurements, but the value obtained is very nearly 60° for each of the six interfacial angles of the vertical zone. Thus the mineral appears to be hexagonal.

Small pyramidal crystals of ordinary quartz in Pure Jharia and Basaria specimens, and white encrustations of colloidal and cryptocrystalline silica in Ghansadih and Tisra specimens, are found deposited in the interstitial spaces between the much bigger black crystals of this interesting mineral. At Basaria big prismatic rock-crystals of ordinary quartz have been found with nodular limonite in the debris from the neighbouring incline of V seam. The new mineral and the ordinary crystals of quartz usually show no sign of interaction with the rock on which they are found ; but in one of the Basaria specimens, a veinlet appears to have branched out from the main vein of the new mineral formed along the joint plane. No ordinary quartz is associated with this mineral in the veinlet.

Microscopic Characters

Under the microscope, the longitudinal sections of the mineral are long and rectangular, sometimes hexagonal (Figs. 3 and 4). They have often somewhat irregular margin with the shaly matrix. They always show one set of cleavage lines, but in addition to this, one or two other, rather indistinct, sets of cleavage lines are often seen in many sections. Dirty brownish clayey and ferruginous matter has been deposited in small patches along the cleavage planes and along certain transverse cracks traversing the section. In Basaria specimens, minute biotite flakes have been observed along certain cracks.

The two refractive indices of the mineral agree in value with those of quartz. The mineral has weak ˙birefringence, the sections showing grey and yellowish polarisation colours of the first order. The mineral shows lamellar structure. The various lamellæ extinguish in slightly different positions, and usually have more or less straight margin parallel to *c*-axis. In some of the fragments from Basaria, simple twinning is observed. The elongation of the mineral is slow, and the extinction angle, Z ∧ C, being undulose, varies from 0° to 5°, especially in Pure Jharia specimens. In convergent light, the prismatic sections show two hyperbolæ which flash in the field of view as the stage is rotated.

Some sections of the mineral show elongated hexagonal outline and three sets of cleavage lines (Figs. 2 and 3). The cleavages are inclined to each other at angles varying from 56° to 66° in different sections. In some of these sections, the three sets of cleavage lines are very nearly inclined at 60° and 120° with each other. Such sections are probably more transverse than longitudinal, and they, therefore, show prismatic cleavage lines. These hexagonal sections show grey polarisation colour of the first order under crossed nicols, and give a uniaxial positive figure in convergent light. In other hexagonal sections, one of the three cleavages is prismatic, whereas the other two may be taken to be rhombohedral, as occasionally a few sections of the mineral show cleavage lines making an angle of about 38° or 67° with the prismatic cleavage, thus corresponding to interfacial angles, $m\,r$ $(10\bar{1}0 : 10\bar{1}1)$ and $m\,z$ $(10\bar{1}0 : 01\bar{1}1)$ respectively. A few crushed fragments of the mineral, mounted on a slide and examined in convergent light confirm that the mineral is optically uniaxial positive.

Chemical Composition

Only in the Pure Jharia and the Basaria specimens, are the mineral blades sufficiently thick to be detached from the rock on which they are deposited. In the former case, however, it was not possible to make the mineral completely free from the ferruginous and clayey matter which occurs as a thin coating over the crystals and also filling the cracks in those blades. This impurity cannot be removed even on boiling the crystal fragments with dilute hydrochloric acid for sometime. A rough chemical analysis of the Pure Jharia specimen, undertaken by Dr. S. C. Niyogy of the University College of Science and Technology, Calcutta, showed that the mineral contained 85·5% SiO_2 and amongst the bases Fe, Mg and Al were the most important. It was first thought that the mineral might be a silicate of these metals, but such a high proportion of SiO_2 is quite abnormal for any such silicate.

The mineral specimens recently discovered in Basaria area, are much purer than the Pure Jharia specimens, their blades are also thicker and, therefore, more easily detachable from the rock than those from the other locality. Dr. Niyogy has kindly carried out a complete chemical analysis of the mineral fragments from Basaria area with the following result :

Per cent.

SiO_2	..	95·78	Spectroscopic analysis shows
MgO	..	2·04	the presence of Co, Ba,
Al_2O_3	..	1·80	and Sr, but the quantities
Fe_2O_3	..	0·14	are too small for estima-
CaO	..	0·37	tion.
MnO	..	0·03	
TiO_2	..	Trace	
	..	100·16	

Discussion

If we assume the mineral to be a silicate, and take Mg and Al for the main bases (as shown in the above analysis), the chemical formula of the mineral works out to be approximately $3 MgO. Al_2O_3. 90 SiO_2$. No silicate of this composition is known to occur. Moreover, it has been observed under the microscope that there is plenty of foreign brownish clayey, limonitic and biotitic matter deposited along the cracks in this mineral. In Pure Jharia specimen, the proportion of Fe_2O_3 was found to be 5·5%, whereas in Basaria specimen the amount of Fe_2O_3 is negligible. In the former case, the mineral specimen analysed is associated with ferruginous shale, whereas the Basaria specimen analysed is associated with carbonaceous shale. Fe_2O_3 may thus be regarded definitely as an impurity in this mineral, derived from the wall-rock.

The presence of 1·51% of MgO, 1·12% of Fe_2O_3 and 0·58% of CaO has been observed in Asmanite, a variety of SiO_2 (vide Dana's System of Mineralogy, sixth edition, p. 193). Asmanite is mixed with bronzite, and this may account for these impurities. Similarly, the deposition of limonite and microscopic flakes of lamellar biotite along some of the cracks of the present mineral, may account for the small percentages of MgO, Al_2O_3 and Fe_2O_3 found in it.

The source of the biotite flakes found in the mineral is not known. Some of the carbonaceous shales (e.g., the carbonaceous shales of Tisra area) in which this mineral has been formed, contain a lot of biotite in addition to muscovite. There are no sills of mica-trap in the immediate

vicinity of any of the localities where this mineral has been discovered, though burnt out-crops of coal or shales may be observed at some places, not far from those localities. There may be some hidden intrusive sills or masses of mica-trap underneath these areas, and the biotite deposited in the cracks of this mineral as well as that formed in the associated carbonaceous shale, might have been ultimately derived from them.

As the mineral resembles quartz in its optical properties as well as in hardness and specific gravity, it has been regarded as a variety of quartz. From the mode of deposition of this mineral along the joint planes of shales and sandstones, its association with ordinary quartz and cryptocrystalline and colloidal silica, and its prismatic rhombohedral habit, it may be inferred that the mineral is of low temperature origin, for high temperature quartz has simple rhombohedral habit (*vide* Winchell's *Optical Mineralogy*, Part II, Second edition, p. 57). But the cleavages, prismatic as well as rhombohedral, shown by this mineral are characteristic of high temperature quartz (*vide* Winchell, *op. cit.*, p. 56).

Further, the presence of biotite flakes observed along a few cracks of this mineral also indicates that the mineral is a variety of high temperature quartz, and that there is possibly some genetic relation between the formation of this mineral and some hidden mica-trap intrusions.

The mica-traps or the so-called 'mica-peridotites' of the Jharia Coal-field are amygdaloidal rocks, occurring as sills. Quartz is one of the most common minerals filling the amygdules in these rocks. At some places, beautiful prismatic and pyramidal rock-crystals, occasionally amethystine, have been collected from the geodes of these traps. If the new mineral were genetically connected with these igneous intrusions, and were deposited by the silica-bearing solutions, emanating from the residual portion of mica-peridotite magma, we should expect to find more of this mineral in these geodes than elsewhere ; but so far this mineral has not been discovered in any of the mica-trap sills.

Hence, we seem to have here a mineral which shows the properties of high temperature quartz, though the source of the solution from which it has been deposited, cannot be definitely attributed to the igneous intrusions of the mica-trap, the only possible source from which silica-bearing solutions have originated. As the crystals of ordinary quartz are also found deposited along the same joint planes and in between the blades of this mineral, the latter should be regarded as a variety, different from the former, and of earlier generation.

Conclusion

In the eastern portion of the Jharia Coal-field, we get a peculiar variety of quartz which is characterised by containing magnesia and alumina as important impurities. In its paragenesis and crystal habit, the mineral resembles low temperature quartz, but considering its well-developed cleavage and association with biotite, it should be regarded as a high temperature quartz. It differs from all the varieties of quartz in showing a characteristic schiller, metal-like lustre and bladed structure.

It is not unusual to give a name to such an interesting and novel variety of mineral. The mineral has been found in the Jharia Coal-measures and investigated in the Geological Laboratories of the Indian School of Mines. The author, therefore, proposes to call this mineral 'Royite' after the name of his colleague, Professor S. K. Roy, in recognition of the wide knowledge which he possesses about the Jharia Coal-measures, the home of this mineral.

Acknowledgments

EXPLANATION OF FIGURES

FIG. 1.   Bladed crystals of "Royite" (grey) with white interstitial patches of colloidal and cryptocrystalline silica in 2 and 3 ×2/5 natural size. 1 from Pure Jharia, 2 from Ghansadih, 3 from Tisra.

FIG. 2.   Elongated hexagonal sections of "Royite" showing three distinct sets of cleavage lines, in contact with ferruginous matrix ×17 ordinary light. Locality—Pure Jharia.

FIG. 3.   Rectangular and hexagonal sections of "Royites". The hexagonal section shows traces of the three cleavage directions, and patches of limonite. Cracks in the rectangular section are filled with opaque ferruginous matter ×17 ordinary light. Locality—Pure Jharia.

FIG. 4.   Rectangular and hexagonal sections of "Royite" showing cleavage lines. The rectangular sections show lamellar biotite along the cracks, and patches of limonite ×17 ordinary light. Locality—Basaria.

FIG. 1
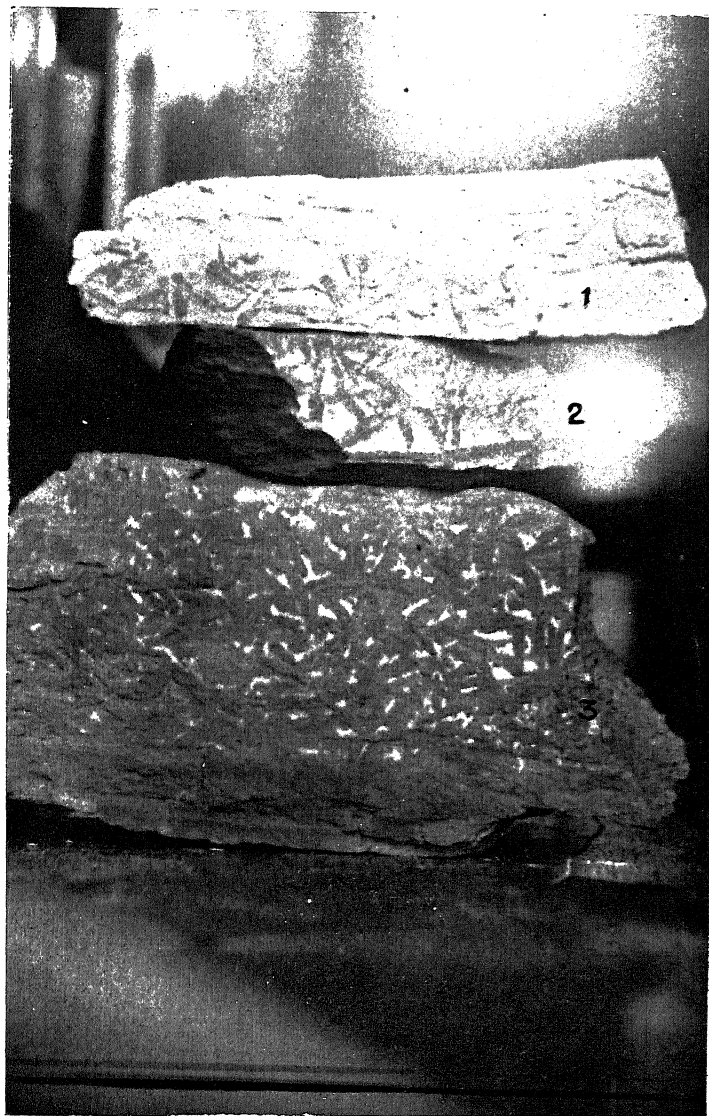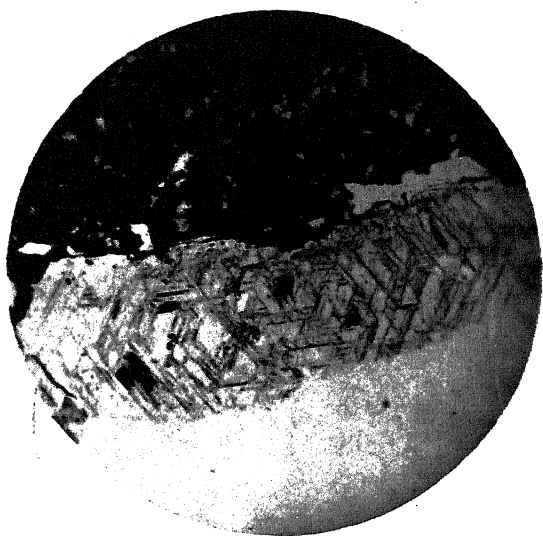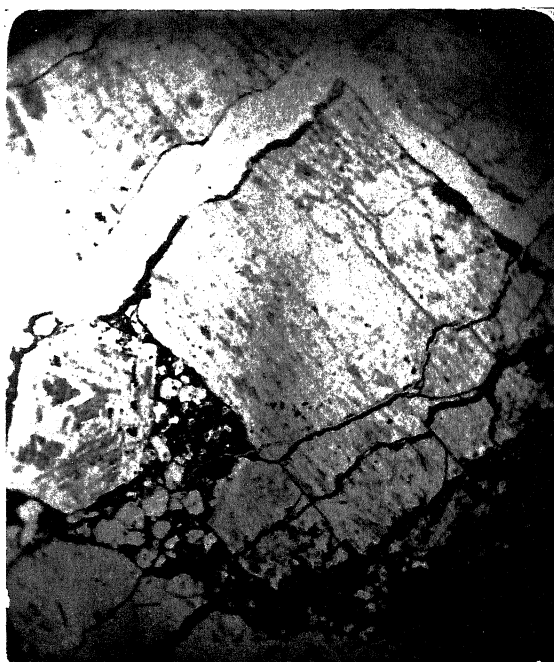
Fig. 4

Fig. 2

Fig. 3

STUDIES IN THE CAPPARIDACEÆ

VIII. The Cytology of *Capparis Zeylanica* Linn., and Related Genera

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CONTENTS

I. *Introduction*

THIS is the genus after which the family is named. It is representative of the sub-family Capparidioideæ. Cytological work on the family is very meagre indeed and that pertaining to this sub-family is confined to chromosome counts in five species of this genus, one of which was by the senior author on *Capparis sepiaria* (Raghavan, 1938). Recently we described the cytology of *Cratæva religiosa*, another member of the Capparidioideæ (Raghavan and Venkatasubban, 1939) and recorded the presence of secondary association. Besides these, there is no cytological work on the Capparidioideæ.

In the present paper haploid chromosome counts have been made of three members of the sub-family for the first time, *Capparis Zeylanica*,

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Cadaba indica and *Mærua arenaria.* In *Capparis Zeylanica,* meiosis has been described in some detail especially as regards secondary association and in *Cadaba indica* material of which was not available in sufficient quantities for a more detailed study, only the meiotic chromosome number has been reported. So also in the other genus.

In all these, it must be said that securing good cytological preparations was a matter of extreme difficulty. It is difficult enough in the Capparidaceæ as a whole, but it would appear to be especially so in this sub-family.

Material for this study was obtained from plants grown in the University Botanical Garden. *Capparis Zeylanica* Linn. which is synonymous with *Capparis horrida* Linn., is a woody climber with prominent recurved thorns which are homologous with stipules. The presence of accessory buds and consequent occurrence of extra axillary flower buds is a feature worthy of note.

Anthers of the right stage of development previously determined by acetocarmine examination, were fixed in Navashin's fluid after prefixation in Carnoy. Fixation of entire flower buds even after the removal of the calyx would not yield any good results. The deletion of prefixation was attended by certain failure to get any preparation worth the name. Embedding was done in paraffin in the usual way after the chloroform technique and Newton's Iodine gentian violet was exclusively used for staining. The average thickness of the sections was about ten microns.

II. Observations

(*a*) *Capparis Zeylanica* Linn.—Stages earlier than First Metaphase were not studied in any detail on account of the extremely small size of the chromosomes. But even at diakinesis it could be clearly seen that the bivalents approximated closely to one another into separate groups. The most prominent feature of the First Metaphase was the occurrence of secondary association. As a matter of fact this phenomenon was so strongly in evidence that hardly a plate existed but exhibited secondary pairing. This is also evidenced by a glance at the accompanying table giving an analysis of the various associations which would reveal that the range of variation of the number of associations is so small and between such high numbers as 7 and 13. The number of bivalents at M I is 20. Figures 1–10 and Pl. IX, Figs. 1–3 show various M I plates showing different degrees of secondary association. Table A gives a summary of the various associations met with.

TABLE A

No. of associations	No. of bivalents in association				No. of cases	Total
	1	2	3	4		
7	8	3	2	..	1 ⎫	2
	7	5	1	..	1 ⎭	
8	4	8	..	..	1 ⎫	8
	6	5	2	..	2	
	5	6	1	..	4	
	7	2	3	..	1 ⎭	
9	3	7	1	..	2 ⎫	6
	4	5	2	..	2	
	5	3	3	..	1	
	2	9	..	..	1 ⎭	
10	2	6	2	..	4 ⎫	8
	3	5	1	1	2	
	4	3	2	1	1	
	3	4	3	..	1 ⎭	
11	1	5	3	..	4 ⎫	6
	2	5	..	2	1	
	2	4	2	1	1 ⎭	
13	1	2	1	3	1	1
					TOTAL ..	31

The maximum association is 3 (4), 1 (3), 2 (2), and 1 (1); and this was met with only once (Fig. 10 and Pl. IX, Fig. 1).

Secondary pairing is solely dependent on the diakinesis position of the bivalents relative to one another, and therefore all bivalents that lie adjacent at diakinesis and which are capable of secondary pairing are so paired at M I. As already indicated at diakinesis itself the bivalents could be seen to be closely approximated and it is these that constitute the secondary groupings at M I.

An interesting feature of this genus not recorded either in *Cratæva* or *Gynandropsis* is that associations of four are very common. Another feature is the association between bivalents of dissimilar sizes (Figs. 6, 10, and Pl. IX, Fig. 1). This would indicate an affinity between non-homologous chromosomes which have some segments in common.

First division is not followed by wall formation. There is an interkinesis when the nuclear membrane is quite in evidence and the chromosomes are distributed more or less peripherally. Interkinesis is of very short

B3
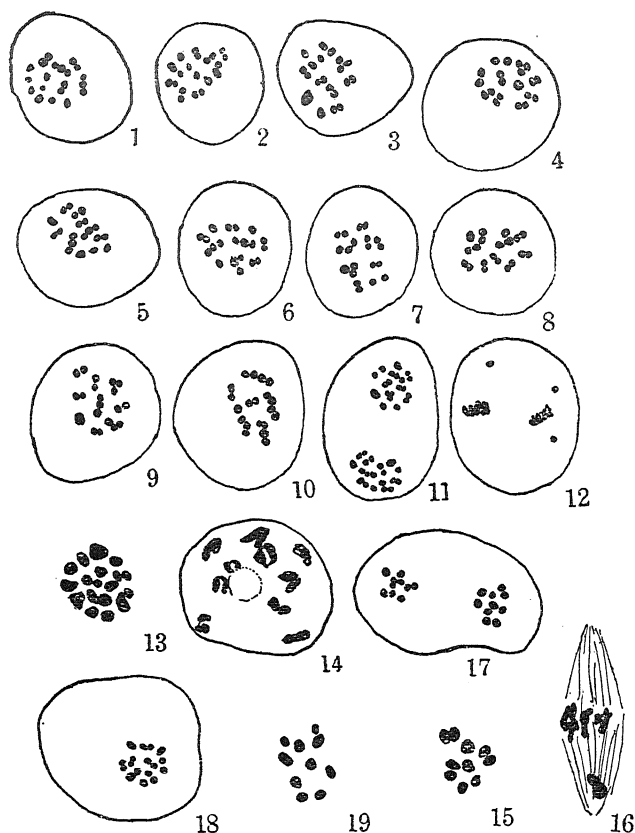
duration and is immediately followed by Second Metaphase when the chromosomes lose their peripheral distribution and arrange themselves as a flat plate. The nuclear membrane has disappeared by this time. Secondary association between chromosomes still persists. Figs. 11 and Pl. IX, Fig. 4, represent the chromosome groupings. The same considerations that apply for the First Metaphase approximation of bivalents would appear to govern the groupings of M II chromosomes also. Enough M II plates were not available to make a comparative study of the groupings at this stage with those of M I. But Catcheside (1937) has made such a statistical study and found that M II plates show various degrees of secondary association covering the same range of types as at M I and in similar proportion.

During Second Metaphase there is not infrequently seen extrusion of variable number of chromosomes into the cytoplasm. Fig. 12 shows three having been extruded. Extrusion of four such bodies is also frequently seen. Presumably these will not be included in the tetrads to be formed and

therefore they be will deficient. The pollen grains, some proportion of them would degenerate. Though division is on the whole normal, tetrads were seen to degenerate in a number of cases. Obviously these degenerations are a result of the deletions referred to.

In a plant growing in a locality close by, degenerations are extensive. There is practically no seed formation and when we investigated the plant it was found that pollen formation was very scarce. Degenerations set in during all stages of pollen development. Even as early as the differentiation of the microsporogenous cells whole anther loculi were seen to degenerate *en masse*. It would therefore appear that in this species there is a tendency towards extensive degeneration.

(*b*) *Cadaba indica* Lamk., *Mærua arenaria* Hook. f. and Thomp., *etc.*— In addition to *Capparis Zeylanica* dealt with in this paper, chromosome numbers of two important genera have been determined for the first time. Fig. 13 shows the p.m.c. of *Cadaba indica* in M I. The haploid number is 18. An interesting feature in this is the varying size of the bivalents indicating that in the somatic complements there should be chromosomes of different sizes.

In *Mærua arenaria* the other genus, the haploid number is 10. Fig. 14 shows a p.m.c. in diakinesis. The 10 bivalents are distributed peripherally. Most of these are of the rodtype. Fig. 15 is an M I plate. Disjunction is normal. Occasionally cases of non-disjunction occur. In Fig. 16 a bivalent is seen to reach the pole earlier without having undergone separation. At M II the 10/10 distribution is almost the rule (Fig. 17) except in rare cases where slight variations are met with obviously due to the non-disjunction mentioned above.

In a previous paper (Raghavan, 1937) the chromosome number of *Cleome Chelidonii*, based upon aceto-carmine smears, was tentatively given as ten. It was not possible then to confirm it by further fixation. Now extensive fixation of the anthers of this species was rendered possible on account of a plentiful availability of material and it is seen that the haploid number is 17 (Fig. 18). It is quite likely that in the previously examined aceto-carmine preparation, the full number could not be counted obviously because of secondary association of bivalents, a phenomenon which has been found to be very widely prevalent in almost all the members of the Capparidaceæ examined so far. It is of interest also to record here that in the closely related *Gynandropsis pentaphylla*, where also the haploid chromosome number is 17, the frequency of occurrence of secondary paired bivalents in groups of 10, was found to be almost at the region of the mode. It is not therefore

unlikely that the few plates examined in aceto-carmine showed the modal groupings and the approximation is so close that groups can easily be mistaken for individual bivalents.

The haploid number of *Cleome viscosa* Linn. was confirmed to be 10 (Fig. 19).

It is interesting that though *Gynandropsis pentaphylla* and *Cleome viscosa* are so strikingly similar morphologically, their chromosome number is so different; whereas *Cleome Chelidonii* whose resemblance to *Gynandropsis* is very much less, shows the same chromosome number. Similarly, *Mærua* a member of the Capparidioideæ and *Cleome viscosa* of the Cleomoideæ exhibit the same chromosome number. This and other irregularities would make it very difficult to accept the primitivity of the arboreal Capparidioideæ. At any rate the evolution of these genera seems to have followed irregular lines.

III. Discussion

(a) *Capparis Zeylanica—a secondary polyploid.*—On the basis of maximum association the gametic constitution of the species may be represented by ·

aaaa

bbbb

cccc

ddd

ee

ff

g

It has already been indicated in the previous papers that seven is likely to be the primary basic number of the family and this would appear to be corroborated by the observations recorded herein. A natural cross between two seven chromosomed parents (one of which had presumably its chromosomes changed structurally by gene mutation, etc.), would lead to the ultimate formation of a tetraploid by amphidiploidy with the somatic constitution a a a'a'–g g g'g', on the basis that the parental genomes that entered into the cross were represented by a–g × a'–g'. If the chromosomes a'–f' undergo reduplication then we get a form with $2n = 40$. The same result could also be obtained if the original seven-chromosomed ancestor (a–g) had been fertilized by the diploid gamete of the a'–g' sister plant and if this were followed by syndiploidy it is likely that a forty-chromosomed plant might have survived by the deletion of a pair of chromosomes through

otic or mitotic aberration. On this assumption the gametic genom of resulting forty-chromosomed species may be represented as:

a a′a′
b b′b′
c c′c′
d d′d′
e e′e′
f f′f′
g′g′ or gg′

evidenced by secondary pairing which is a strong indication of the estral homology between chromosomes a and a′, b and b′, etc., we should ect a maximum association of seven consisting of six threes and one two 3) and 1 (2)]. But such an association is not to be found and groups our are very common. This makes one infer that structural changes have ved a part in the evolution of the species in addition to polyploidy; this be explained by structural changes chiefly in the nature of reciprocal slocation as having taken place between different chromosomes. The ected somatic constitution may be represented as:

a aa′a′a′a′
b bb′b′b′b′
c cc′ c′c′c′
d dd′d′d′d′
e ee′ e′e′e′
f ff′ f′f′f′
 g′g′g′g′ or gg g′g′

egmental interchange takes place between a and e, b and f, and g and c omosomes, then the result will be:

a (ae) a′a′ a′a′
b (bf) b′b′ b′b′
c (cg) c′c′ c′c′
d d d′d′ d′d′
e (ea) e′e′ e′e′
f (fb) f′f′ f′f′
g (gc) g′g′

account of the new structural homology thus introduced, naturally bi-ents a (ae) and e (ea) will be secondarily associated. Similarly b (bf) f (fb) and so on. The result of this would be three groups of four, e group of three and two groups of two and a single bivalent unassociated. is means that the affinity between a (ae) and a′a′ bivalents has not been

impaired by this structural change. The frequent association of bivalents of dissimilar size is a clear indication of an affinity between non-homologous chromosomes which have some segments in common.

As an alternative the following method of origin of the forty-chromosomed species may be considered:

Eight of the chromosomes of the tetraploid may be lost through deletion, say $d'-g'$. Supposing the constitution of this 20-type be X, gene mutation or structural change may modify this type to produce a species of the constitution X'. Amphidiploidy is likely to occur when X and X' are crossed, so that a new type with 40 chromosomes arises. But here the maximum association should be 3 (4) and 4 (2), assuming as we did the deletion of the $d'-g'$ chromosomes. But instead we get 3 (4), 1 (3), 2 (2) and 1 (1). This can only be explained by reciprocal translocation as having taken place. Supposing the parental gametic genomes were of the following constitution:

$$X'$$

$$X$$

$$A_2A_2\ A_3A_3$$
$$B_2B_2\ B_3B_3$$
$$C_2C_2\ C_3C_3$$
$$D_3D_3$$
$$(D_2-G_2 \text{ deleted})\ E_2E_3$$
$$F_3F_3$$
$$G_3G_3$$

X

$$AA\ A_1A_1$$
$$BB\ B_1B_1$$
$$CC\ C_1C_1$$
$$D_1D_1 \quad (\text{D–G chromo-}$$
$$E_1E_1 \quad \text{somes deleted})$$
$$F_1F_1$$
$$G_1G_1$$

A cross between these would result in a species having the following constitution:

$$AA\ A_1A_1\ A_2A_2\ A_2A_3$$
$$BB\ B_1B_1\ B_2B_2\ B_3B_3$$
$$CC\ C_1C_1\ C_2C_2\ C_3C_3$$
$$D_1D_1\ D_3D_3$$
$$E_1E_1\ E_3E_3$$
$$F_1F_1\ F_3F_3$$
$$G_1G_1\ G_3G_3$$

On this basis one would expect a maximum association of 3 (4) and 4 (2). If however reciprocal translocation takes place between D_1 and E_1 chromosomes, then we get, D_1 (D_1E_1) and E_1 (E_1D_1) and on the basis of homology between D_1 and D_3 chromosomes which we have assumed, we may get a group of three: D_3D_3, D_1 (D_1E_1), E_1 (E_1D_1). E_3E_3 would be left alone. F_1 and F_3, G_1 and G_3 will form a group of two.

We find therefore that on either of these assumptions, structural changes, chiefly reciprocal translocation, would appear to have played a prominent part in the evolution of the species. In the former almost all the chromosomes were involved except the D chromosomes. In the latter, only a few chromosomes, D_1 and E_1 chromosomes would appear to have been affected structurally. Since, however, it is very common we find in groups of four, association of bivalents of dissimilar size, it is likely that it is these b.f. chromosomes that have undergone structural changes and as such the first assumption is more tenable.

(*b*) *Secondary Association — its limitations as a guide to polyploidy.*— Evidence from secondary association alone cannot be regarded as conclusive in respect of ancestral homology and consequently of the basic number. One of the most common factors which would make secondary pairing unreliable, unaided by other evidence, is that structural changes of chromosomes may hamper this phenomenon. Structural changes of the homologous chromosomes may have taken place to a great extent in polyploids and as a consequence the degree of affinity required to cause attraction may not be present. Or translocations, simple or reciprocal, which are very common factors in the evolution of new species may give rise to higher associations, so that the basic number inferred from observed secondary pairing may be erroneous. Numerical differences cause changes in frequency; high numbers of chromosomes tend to reduce the chance of association between similar chromosomes. Large size of the chromosomes appears to inhibit secondary pairing since it is seen generally only in organisms with small chromosomes.

In the present paper we have explained the maximum association on the basis of segmental interchange. Unaided by any previous knowledge it may not be proper to conclude that the maximum grouping represented the basic number. But since chromosome behaviour in two other genera, representative of the two sub-families, has already been studied and ample evidence let in to show that seven was likely to be the primary basic number of the family, the maximum association seen in this important genus has been interpreted in the way it has been done. The noteworthy fact is that reciprocal translocation and other structural changes which have undoubtedly played an important rôle in the evolution of the species have taken place in such a manner as to keep up the original number, though the groupings have undergone corresponding changes.

If chromosome interchange had taken place, then the absence of ring formation is rather hard to explain. But since the chromosomes are very

small segmental homology manifests itself in an association of dissimilar chromosomes rather than in actual ring formation. Moreover it is not absolutely necessary that reciprocal translocation should be always followed by ring formation. A few cases have been reported where this has failed to occur. For instance, Clarke and Anderson (1935) have shown that in Maize chromosome interchange takes place without the external evidence of ring formation.

Heilborn (1936) considers that no credence should be placed on secondary association as indicative of ancestral homology and that it is a purely physical phenomenon, that chromosomes of equal size are associated or tend to be associated irrespective of their homology. According to him it is not a specific attraction or pairing between homologous parts of chromosomes but the parallelism of the associated chromosomes is mechanically induced through the polarity of the nuclei. Flowik (1938) has shown how this assumption is untenable so far as the genus *Carex* is concerned. In this paper additional evidence is to be found for not accepting the hypothesis. There is clear evidence of bivalents of different sizes associating. Primarily a result of ancestral homology, structural changes have also played a prominent part in this. There is also evidence of pairing of chromosomes of dissimilar size as could be seen from side views of metaphases. Observations of a similar nature have been made in other genera also. For instance, in *Cicer*, Iyengar (1939) has recorded the association between a short rod bivalent and a long one. Richharia (1937) has made a similar observation in the genus *Brassica*.

(c) *Phylogenetic Considerations.*—The chromosome numbers of the genera and the species so far investigated in the Capparidaceæ form an irregular series. Polyploidy, structural changes, meiotic and mitotic aberrations have undoubtedly played an important part in the evolution of the species. The numbers known so far are so few that generalizations at this stage may not be quite warranted. A few remarks can, however, be made regarding the distribution of the chromosome numbers in the various genera. The Capparidaceæ are almost entirely tropical, a few sub-tropical and rarely temperate in their distribution. From the chromosome list now available one finds that generally speaking, the sub-tropical and temperate genera have greater chromosome numbers than the tropical ones. This is in keeping with observations made previously in a few families. For instance, in the Cactaceæ, Stockwell (1935) found that the *Opuntias* which were the most northern of the *Cacti* had higher chromosome numbers than the rest. Even among the same genus the more northern species had a greater chromosome number than the southern ones. Similarly Hagerup (1928) observes "it is worth noting
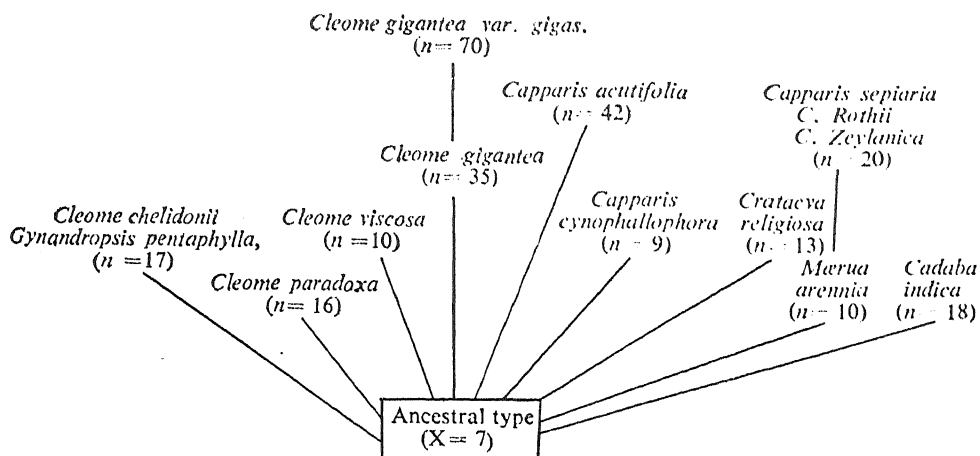
that among these four pairs of species (including *Empetrum nigrum* and *E. hermaphroditum*) those with the higher chromosome numbers are always the ones growing farther north ".

It is also likely that the original home of the Capparidaceæ was the Tropics. There would appear to be a correlation between distribution and polyploidy and it is quite conceivable that some of the sub-tropical species like *Cleome gigantea*, *C. g. var. gigas*, *Cleome spinosa*, etc., have essentially by polyploidy conquered new territories. Nawaschin (1929) says, " through changes in the rate of development a polyploid individual may acquire the ability of withstanding different climatic conditions and as a consequence penetrate into new territory." Hagerup (1933) also says, "polyploid forms may be ecologically changed so as to grow in other climates and formations where the diploid forms will not thrive ". Clearly the species mentioned above are polyploids.

From purely cytological evidence—and even that is very meagre in this family—it is difficult to assemble the genera phylogenetically, not only because chromosome bumbers are not absolutely diagnostic but also the numbers known so far are very irregular. But a few general remarks may not be out of place nonetheless. There is to be seen, though not very apparent, an increase in chromosome numbers as one passes from the taxonomically more primitive to the more advanced Capparidaceæ. Whether this is accompanied by any marked decrease in chromosome size, as it usually is the case, cannot be said. The chromosome numbers known up-to-date, more or less confirms the taxonomic evidence that the genus *Corparis* is comparatively primitive. It must be said that it is difficult to determine the exact relations of the more primitive genera with one another. The cytological data available may, however, be utilized for indicating the broad sectional relations rather than for the alignment of species.

It has already been suggested that seven is likely to be the primary basic number of the family and from this a number of secondary basic numbers have arisen and the various genera represent different balances of these numbers. In this allopolyploidy as indicated by secondary association has played a prominent part. A species of *Capparis* shows the lowest chromosome number known in the family ($2n = 18$) and conjoint with evidence available on morphological and taxonomical grounds, *Capparis* as representative of the sub-family Capparidioideæ is to be regarded as more primitive. We find, however, that almost the same secondary basic numbers are to be found in the two sub-families, Cleomoideæ and the Capparidioideæ and as such the evolution of the genera in the two tribes may be regarded

as representing parallelism at least so far as the chromosome numbers are concerned. This may be represented diagrammatically in a rough manner as follows:

Cleome gigantea var. gigas.
(n = 70)

Capparis acutifolia
(n = 42)

Capparis sepiaria
C. Rothii
C. Zeylanica
(n = 20)

Cleome gigantea
(n = 35)

Cleome chelidonii
Gynandropsis pentaphylla,
(n = 17)

Cleome viscosa
(n = 10)

Capparis
cynophallophora
(n = 9)

Crataeva
religiosa
(n = 13)

Cleome paradoxa
(n = 16)

Maerua
arenaria
(n = 10)

Cadaba
indica
(n = 18)

Ancestral type
(X = 7)

IV.   Summary

The haploid chromosome numbers of the following have been determined for the first time;—*Capparis Zeylanica* Linn. = Twenty (20); *Cadaba indica* Lamk. = Eighteen (18); *Maerua arenaria* Hook. f. and T. = ten (10). The chromosome number of *Cleome viscosa* Linn. is confirmed to be ten (10) and that of *Cleome Chelidonii* Linn. is seventeen (17) and not ten as previously reported.

Secondary association is reported in *Capparis Zeylanica* Linn. and the previous finding that the primary basic number of the family, 7, is supported by observations made herein.

Evidence is found to show that polyploidy as well as structural changes of chromosomes have played an important part in the evolution of the species. The association of 4 bivalents is interpreted on this basis.

Secondary association and its limitations as the sole factor in determining ancestral homology are discussed in the light of the present findings and of the data gathered previously on other genera of this family.

Some general remarks are made on the distribution of chromosome numbers in this family and a tentative scheme formulated to indicate phylogenetic evolution of some of the important genera.

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LEGEND TO TEXT-FIGS. 1–19

(All figures have been drawn at a magnification of ca 3,300.) Figs. 1–10. First Metaphase plates of *Capparis Zeylanica* Linn., showing varying degrees of secondary association.

FIG. 1 shows an association of   2 (3) 3 (2) 8 (1)

FIG. 2   ,,   ,   8 (2) 4 (1)

FIG. 3   ,,   ,,   1 (3) 6 (2) 5 (1)

FIG. 4   ,,   ,,   1 (3) 7 (2) 3 (1)

FIG. 5   ,,   ,,   2 (3) 5 (2) 4 (1)

FIG. 6   ,,   ,,   3 (3) 4 (2) 3 (1)

FIG. 7   ,,   ,,   2 (3) 6 (2) 2 (1)

FIG. 8   ,,   ,,   2 (4)   5 (2) 2 (1)

FIG. 9   ,,   ,,   3 (3) 5 (2) 1 (1)

FIG. 10   ,,   ,,   3 (4) 1(3) 2 (2) 1 (1) Maximum association.

FIG. 11.—M II showing the 20/20 distribution; note the persistence of secondary association

FIG. 12.—M II showing the extrusion of three chromosomes.

FIG. 13.—First Metaphase plate of *Cadaba indica* Lamk., showing 18 bivalents.

FIG. 14.—P. M. C. of *Mærua arenaria* Hook. f. and T. in diakinesis.

FIG. 15.   ,,   ,,   ,,   in first metaphase, 10 bivalents.

FIG. 16.   ,,   ,,   ,,   in first metaphase, note one of the bivalents
                                                                 reaching the pole earlier.

FIG. 17.   ,,   ,,   ,,   M II   Note the 10/10 distribution.

FIG. 18.—P. M. C. of *Cleome Chelidonii* Linn. f., in first metaphase showing 17 bivalents.

FIG. 19.   ,,   *Cleome viscosa* Linn., in M I showing 10 bivalents.

EXPLANATION OF PLATE IX

FIG. 1.—Microphotograph of P.M.C. of *Capparis Zeylanica* Linn., showing maximum association·
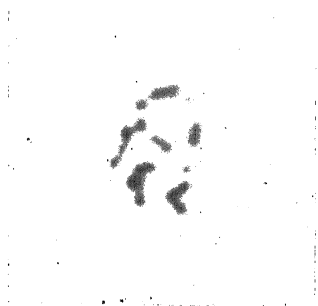   Same as Text-Fig. 10.

FIG. 2.—Microphotograph of P.M.C. of *Capparis Zeylanica* Linn., showing the same association
        as Text-Fig. 9.

FIG. 3.—Microphotograph of P.M.C. of *Capparis Zeylanica* Linn., showing an association of
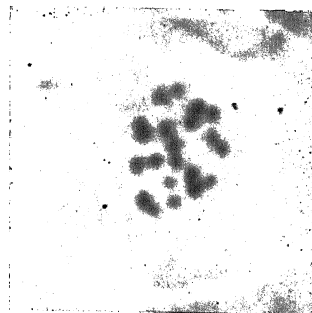   3 (3) 5 (2) 1 (1).

FIG. 4.—Microphotograph of second metaphase showing secondary association persisting.
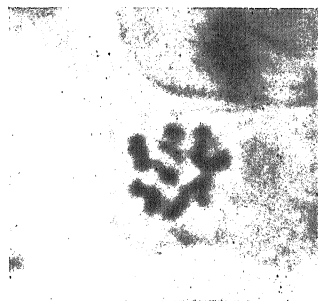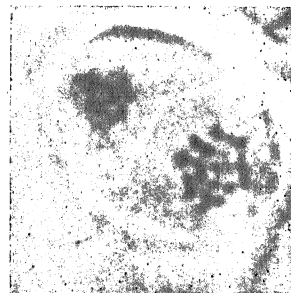
1

2

3

4

AN ANALYSIS OF ONE HUNDRED NORMAL ELECTROCARDIOGRAMS

(Boys aged 5 to 15 years)

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Object of Study

The present work was undertaken with a view to determine normal electro-cardiographic standards for Indian subjects. Though electrocardiographic standards have already been worked out by various workers, they are often based on insufficient numbers of cases and apply to European or American subjects only. No reliable figures or standards exist at the present day for Indian subjects. The present investigation allows us to present electro-cardiographic standards, based on a fairly large series of normal Indian subjects, for the use of practitioners in India. Generally accepted standards, obtained by workers in Europe and America, are also presented for the sake of comparison.

Selection of Material

Electrocardiograms (the three standard leads) were taken of well over one hundred school-boys at the Bharda New High School, Bombay. Only boys between the ages of 5 and 15 years were selected for the study. After excluding all subjects with any suggestion of weakness or disease of the heart, hundred normal students were included in this study; their electro-cardiograms were subjected to a detailed study and the results tabulated as far as possible. All the tracings were taken with the aid of a Victor (G.E.C.) portable electrocardiograph, with the subject at rest and in a sitting position; measurements given in the text were made according to the standard method.

The P Wave

The first exhaustive study of the P Wave or the "Auricular Wave" was made by Lewis and Gilder[8] in 1912. The P Wave commences just before the rise of intra-auricular pressure that occurs during auricular systole

and ends sometime before actual completion of the auricular systole. This wave represents *early* activity of the muscle-fibres of the auricles.
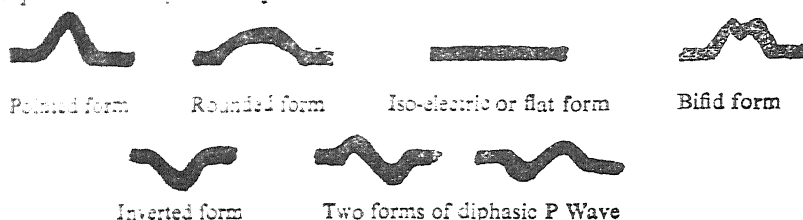

Pointed form   Rounded form   Iso-electric or flat form   Bifid form

Inverted form   Two forms of diphasic P Wave

FIG. 1

Different Forms of P Wave

Form of the P Wave.—The P Wave displays a variety of forms. (Fig. 1). Though usually described as a smooth rounded elevation, the pointed form of P Wave is no less common than the rounded form. In the present series of cases, the pointed form of P Wave was more frequently observed than the rounded form (the percentage frequencies being 39% and 30% respectively). The higher incidence of the pointed form of P was observed in all the three standard leads of the electrocardiogram (Table I). In the series of normals reported by Hoskin and Jonescu,[8] the pointed form of P was much more prevalent than the rounded form (the percentage frequencies being 52·6% and 30·6% respectively). In the series of two hundred normals reported by Shipley and Hallaran,[15] rounded P waves were observed in 67% of records and the pointed form in 33%.

TABLE I

Percentage Frequencies of the Different Forms of P Wave (100 Cases)

Description of P Wave	Lead I	Lead II	Lead III
1. Pointed form	42	49	27
2. Rounded form	38	31	20
3. Pointed and rounded	6	9	6
4. Flat or iso-electric	4	1	15
5. Bifid form	6	6	5
6. Inverted form	2	0	14
7. Diphasic form	2	4	13

In some records, the form of the P Wave is half-way between the pointed and the rounded forms; in some, the P Wave form is inconstant and shows transition forms between the pointed and rounded varieties.

The above-mentioned two groups of cases have been classed together as " the pointed and rounded form " of P Wave, in the present paper. The tendency for the P Wave to vary in form from cycle to cycle in perfectly healthy individuals is difficult to explain; inconstancy of form probably depends, to some extent at least, on the inconstancy or variability of the path pursued by the excitation wave in the substance of the auricular musculature. The alteration in form may also be dependent to some extent on the phases of respiration.

Flat or iso-electric P Waves were observed in Lead I in 4% of records, in Lead III in 15% of records and only once in Lead II. Inverted or negative P Waves were noted in 2% of records in Lead I, and in 14% of records in Lead III; they did not occur in Lead II at all. In the series of Hoskin and Jonescu,[3] inverted P Waves were observed in Lead III only, in 12% of cases.

According to Pardee,[13] diphasic P Waves may occur in normal subjects in Lead III and occasionally also in Lead I; he does not mention the occurrence of diphasic P Waves in Lead II. In my series, diphasic or bi-directional P Waves were seen in Lead I in 2% of cases, in Lead II in 4% of cases and in Lead III in 13% of cases.

From the above figures, it will be observed that in 29% of records, there was no deflection of the P Wave above or positive to the base-line, in Lead III.

Notching of P Waves.—This was once regarded as a reliable sign or indication of auricular hypertrophy. However, in later years, various observers have independently observed their occurrence in a fair number of perfectly normal records. Thus in the series of 52 normal records, studied by Lewis and Gilder,[8] 17 showed notching of P in one or more leads; P_1 was notched in 2 cases, P_2 in 17 and P_3 in 10 cases. Notching of P was present in 30% of the two hundred records reported upon by Shipley and Hallaran.[15] In my series, notching was observed in one or more leads in 35 records (*i.e.*, 35%); in only one case was notching of P found in all the three standard leads. Notching of P_1 was noted in 14 records, of P_2 in 22 records and of P_3 in 18 records. In 18 records, there was notching of P in two leads; notching of P_1 and P_2 in 7 records, of P_1 and P_3 in 3 records and of P_2 and P_3 in 7 records. In 17 records, notching of P was confined to one lead only; notching of P_1 in 3 cases, of P_2 in 7 cases and of P_3 in 7 cases.

That notching of P does not necessarily signify auricular hypertrophy is obvious from the high incidence of such P Waves in my series of perfectly normal records. In Pardee's[13] opinion, " notching must be accepted as

one of the normal variations of the P Wave"; such is indeed the opinion of the majority of present-day cardiologists. The phenomenon of P Wave notching has been lucidly dealt with by Pardee;[13] according to him, the P Wave represents a synchronous overlapping of two electrical effects, one from each auricle; if, for some reason, the path of the excitation wave in one auricle becomes tortuous or deviated, then the two electrical effects fail to coincide and notching of the P Wave ensues.

Notching in P Waves of large Amplitude.—It has been shown by Shipley, Hallaran,[15] Hoskin, Jonescu[3] and others that the association in the P Wave of a large amplitude (over 2 mm.) with notching, cannot be regarded as a normal variation. A combination of, notching and large amplitude should suggest the existence of mitral stenosis; it was not observed in a single case of Hoskin's and Jonescu's.[3]

In my series of normals, there were three records with large and notched P Waves; the amplitudes of P in the 3 cases were 2·3 mm., 2·5 mm. and 2·6 mm. respectively; there was no evidence of mitral disease in any of these three cases. Such has been my experience in England also; on several occasions, I have come across large P Waves with notching in individuals devoid of all symptoms and signs of mitral valve disease. It is possible that in a small percentage of normal records (3% in my series) there may be an association of notching and large amplitude of P even in the absence of mitral stenosis.

Relation of T to P Waves.—A relationship was observed between the P and the T waves by Hoskin and Jonescu[3] and by Shipley and Hallaran.[15] When P was inverted, the T Wave was also inverted and when P was diphasic, T was also diphasic. A study of my records supports the first half of the above contention that inverted P Waves are always accompanied by inverted T Waves. In every single case where the P Wave was inverted, the corresponding T Wave was also inverted. This rule does not however hold the other way about; T-Wave inversion is not necessarily preceded by inverted P Waves; cases of T Wave inversion are common, where the accompanying P Waves are normal and upright.

With regard to diphasic P Waves, my results are not in agreement with those of the above authors.[3,15] On considering diphasic P_3 Waves, the corresponding T_3 Waves were not found to display any constant shape or direction. T_3 was found to be diphasic in 11%, flat in 11%, upright in 11% and inverted in 67% of cases with diphasic P_3 Waves.

With flat or iso-electric P_3 Waves, I found T_3 flat or iso-electric in 55% inverted in 35% and upright in 10% of such cases.

Amplitude or Height of the P Wave.—According to Pardee[13] the amplitude of the P Wave should be between 1 and 2 mm. in that lead in which it shows the maximum excursion; in records, where one lead is iso-electric or diphasic, the amplitude in the other leads being about equal, the value of P should be between 0·9 mm. and 1·8 mm. (Pardee). The average amplitude of P worked out by Hoskin and Jonescu[3] in fifty normal women students, was 1·74 mm. (including Lead IV); the maximum and minimum deflections of P in their records were 5·0 mm. and 0·5 mm. respectively.

After a study of electrocardiograms in 52 healthy subjects, Lewis and Gilder[8] gave the following average values of P in the three standard leads. In Lead I, 0·52 mm.; in Lead II, 1·16 mm.; in Lead III, 0·81 mm.

Effect of Sex on Amplitude of P Wave.—Shipley and Hallaran[15] found the average amplitude of P higher in males than in females, the average values for P_2 in the two sexes being 1·41 mm. and 1·30 mm. respectively. In children ranging in age from 3 weeks to 12 years, Burnett and Taylor[2] (1936) found higher values for P in girls than in boys.

Effect of Age on Amplitude of P Wave.—Burnett and Taylor[2] state that though P Waves show higher and lower deflections in children than in adults, the average value of P shows little variation with age. They give the upper and lower limits of P in children as 2·5 mm. and 0·5 mm. respectively.

Recently, Hoskin and Jonescu[3] found much higher values for P Wave amplitude than previous investigators. In my series of 100 normal schoolboys, the average value for P, taking the three standard leads into consideration, was 0·90 mm. The average value for Lead I was 0·84 mm., for Lead II 1·18 mm. and for Lead III 0·67 mm. The highest values for P Wave amplitude were observed in Lead II (as previously shown by various workers).

Taking the three standard leads into consideration, maximum values for P were observed in Lead II in 63% of cases, in Lead I in 26% and in Lead III in 7%; in other words, practically two-thirds of all records show maximum values for P in Lead II. In 6% of records, P_1 and P_2 were equal in amplitude and in 3%, P_2 and P_3 were equal in amplitude.

Range.—In Lead I, the height of the P Wave ranged from 0·0 to 2·1 mm.; in Lead II, from 0·0 to 3·1 mm.; in Lead III, from − 1·0 to 3·1 mm.

Our analysis of the amplitude of the P Wave in the three leads is reproduced in Table II.

TABLE II

Amplitude of the P Wave (Percentage Frequencies)

Amplitude range (in mm.)	Lead I	Lead II	Lead III
−1 to 0	0	0	6
0 to 1	63	40	62
1 to 2	34	43	27
2 to 3	2	15	3
3 to 4	1	2	2

Duration of P Wave.—The average duration of the P Wave is 0·09 sec. according to Shipley and Hallaran[15] and 0·076 sec. according to Hoskin and Jonescu[3]. The latter authors found the duration of the P Wave shorter in women than in men.

In my series, the mean value of P for the three standard leads was 0·08 sec. The average values of P Wave duration for the individual leads were: 0·079 sec. for Lead I, 0·088 sec. for Lead II and 0·073 sec. for Lead III.

The duration of the P Wave varied from 0·04 to 0·14 sec. in Lead I and from 0·04 to 0·12 sec. in Leads II and III. The longest duration of P in my series was 0·14 sec., observed twice in Lead I only. Further details on P Wave duration are reproduced in Table III.

TABLE III

Duration of the P Wave in 100* Cases (Percentage Frequencies)

Duration of P Wave (in seconds)	Lead I	Lead II	Lead III
0·04	5	2	9
0·05	12	5	14
0·06	11	11	12
0·07	15	10	10
0·08	21	15	16
0·09	11	18	12
0·10	9	16	6
0·11	5	8	8
0·12	2	12	3
0·13	3	0	0
0·14	2	0	0

* The duration of the P Wave could not be determined in four cases in Lead I, in three cases in Lead II and in ten cases in Lead III.

P–Q Duration

This is measured from the end of P to the beginning of the Q deflection. Hoskin and Jonescu[3] found the following mean values for P–Q duration in the three leads: Lead I = 0·06 sec.; Lead II = 0·06 sec.; Lead III = 0·07 sec.

In my series, the average value of P–Q duration for the standard leads was 0·062 sec. The mean values for the individual leads were as follows: Lead I = 0·052 sec.; Lead II = 0·061 sec.; Lead III = 0·072 sec.

Range.—In Leads I and II, the P–Q duration varied from 0·01 to 0·12 sec., and in Lead III from 0·02 to 0·12 sec. The P–Q duration was found to be between 0·04 and 0·08 sec. in as many as 77% of records in Lead I, in 82% in Lead II and in 58% in Lead III.

Maximum values for P–Q duration were found in Lead I, 18 times, in Lead II, 29 times and in Lead III, 72 times. A P–Q duration constant in the three leads, was discovered only once. Maximum values for P–Q duration were observed in two leads in 17 records; 5 times in Leads I and II, 6 times in Leads I and III and six times in Leads II and III.

The results of our analysis of the P–Q duration are reproduced in Table IV.

TABLE IV
Duration of P–Q in 100* Cases (Percentages)

Duration of P–Q (in seconds)	Lead I	Lead II	Lead III
0·01	1	1	0
0·02	3	3	2
0·03	9	3	2
0·04	23	16	6
0·05	21	13	9
0·06	18	22	12
0·07	8	17	9
0·08	7	14	24
0·09	3	2	8
0·10	1	5	12
0·11	0	0	3
0 12	2	2	2

* P–Q duration could not be determined in Lead I, four times, in Lead II, twice and in Lead III, eleven times.

Rustom Jal Vakil

P–R Interval

This is measured from the beginning of the P Wave to the beginning of the Q R S complex, irrespective of whether the initial deflection of this complex is Q. R or S. The P–R interval represents the auriculo-ventricular conduction time. The most correct measurement of the P–R interval is in that lead in which it is longest; this is usually the case in Lead II.

Lewis and Gilder[5] (1912) gave the normal limits of the P–R interval as 0·13 sec. and 0·21 sec. Pardee[16] gives 0·16 sec. as the average duration of the P–R interval. According to Paul White,[17] the average P–R interval is 0·16 sec. in adults and 0·125 sec. in infants and children; the upper limit of normal is 0·20 sec. in adults and 0·18 sec. in infants and children. The duration of the P–R interval ranged from 0·09 to 0·21 sec. in the series reported by Hoskin and Jonescu[8]; they gave 0·13 sec., 0·14 sec. and 0·15 sec. as the average values for the P–R interval in Leads I, II and III, respectively.

In my series of normals, the value of the P–R interval ranged from 0·07 to 0·21 sec. taking all leads into consideration. The mean values for the three leads were: Lead I = 0·136 sec.; Lead II = 0·144 sec. and Lead III = 0·144 sec. The average figure for all leads was 0·142 sec.

Tables V and VI give our analysis of the P–R interval in a tabulated form.

The P–R interval tends to be shorter in children than in adults (the mean values being about 0·14 sec., and 0·16 sec. respectively), the difference in mean values being about 0·02 sec., i.e., 1 50 sec.

TABLE V
Duration of P–R Interval in 100* Cases
(*Percentages*)

Time Range (in seconds)	Lead I	Lead II	Lead III
0·06 to 0·08	0	1	2
0·08 to 0·10	8	2	1
0·10 to 0·12	18	15	14
0·12 to 0·14	32	29	29
0·14 to 0·16	15	27	15
0·16 to 0·18	12	11	17
0·18 to 0·20	5	6	5
0·20 to 0·22	3	—	6

* The P–R interval could not be determined in Lead I, seven times, in Lead II, twice and in Lead III, eleven times.

TABLE VI

Duration of P–R Interval in 100* Cases (Percentages)

P–R Duration (in seconds)	Lead I	Lead II	Lead III
0·07	0	:	2
0·08	3	0	1
0·09	5	2	0
0·10	2	5	4
0·11	7	4	7
0·12	19	13	11
0·13	11	11	10
0·14	21	16	17
0·15	0	13	4
0·16	11	14	15
0·17	4	4	4
0·18	6	6	6
0·19	1	1	2
0·20	2	4	3
0·21	1	4	3

*The P–R interval could not be determined in Lead I, seven times, in Lead II. twice and in Lead III, eleven times.

P–R Interval in the Lead of Largest Measurement.—Since the P–R Interval represents the auriculo-ventricular conduction time. it is obvious that the longest P–R interval in any record (in whatever lead that may be), is the most correct measurement. The hundred records in my series of normals were examined from this point of view. Maximum P–R values were observed 35 times in Lead I, 56 times in Lead II and 45 times in Lead III. The P–R interval was identical in the three leads in only one record. Maximum values for P–R interval were shared by two leads on 30 occasions: 13 times in Leads I and II, 6 times in Leads I and III and 11 times in Leads II and III.

Values for the maximum P–R duration ranged from 0·09 sec. to 0·21 sec. in the hundred records, the average value being 0·153 sec. Further details about maximum P–R values are reproduced in Table VII.

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TABLE VII

Maximum P-R Interval Values in 100 Cases (Cases)

Range of Duration in seconds	Lead I	Lead II	Lead III
0·08 to 0·10	3	4	3
0·11 to 0·13	6	11	9
0·14 to 0·16	19	28	18
0·17 to 0·19	5	11	7
0·20 to 0·22	2	6	4

P–R Level or the Auricular T Wave.—The line of the record between the end of the P Wave and the beginning of the Q R S complex is seldom entirely iso-electric in all the three leads. There is usually a mild degree of deflection or deviation of this line from the zero-line or base-line, practically invariably in a downward direction. This deflection is caused by auricular contraction and corresponds to the T Wave of the ventricular complex; hence it is often known as the Auricular T Wave.

In Pardee's[13] series of twenty-six normal records, the P–R level was deflected downwards in all three leads in 12 records, deflected downwards in two leads in 12 records and in the remaining two records, there was no deflection in any lead. The maximum deflection noted by Pardee was 2·5 mm. At present, no clinical significance is attached to the P–R deflection.

In my series of 100 records, a downward deflection of the P–R interval was observed in all three leads in 24 records (24%) and in two leads in 48 records (48%); deflection of the P–R level confined to one lead only was observed on 14 occasions only (14%). In 13 records (13%), there was no deflection of the P–R interval in any lead.

The deflection of the P–R level from the iso-electric line was measured in the hundred records, in each lead. The deflection, which was deflection in all cases, ranged from 0·0 to 2·0 mm. The mean value of P–R deflection (downwards) for all leads was 0·27 mm. In Lead I, P–R deflection ranged from 0·0 to 1·2 mm. with an average figure of 0·21 mm.; in Lead II, it ranged from 0·0 to 2·0 mm., with an average of 0·38 mm.; in Lead III, the range was 0·0 to 1·0 mm., with an average figure of 0·24 mm.

Further particulars about P–R level deviation are incorporated in Table VIII.

TABLE VIII

Deflection of P–R Level in 100 Cases* (Percentages)

Range of Deflection (in mm.)	Lead I	Lead II	Lead III
0 to 0·3	73	50	62
0·4 to 0·7	21	34	33
0·8 to 1·1	5	13	-
1·2 to 1·5	1	2	0
1·6 to 1·9	0	0	0
2·0 to 2·3	0	1	0

* Deflection of P–R was not observed at all in an upward direction or positive to the base-line ; in all cases where the P–R level failed to coincide with the iso-electric line, the deflection was in a downward or negative direction.

Q R S Complex or Group

This is a complex group made up of three peaks or waves, Q, R and S, which are more or less independent of each other. Absence of Q or S is by no means infrequently observed in perfectly normal records.

Duration of Q R S Complex.—This represents the time consumed by the contraction to spread throughout the ventricles. It is obtained by measuring the time from the beginning of the initial ventricular deflection (Q or R, as the case may be) to the end of the final deflection of the Q R S group (R or S, as the case may be).

Commonly accepted standards for the Q R S duration in adults and in children are reproduced in Tables IX and X, respectively. The duration of Q R S tends to be shorter in children than in adults.

In my series of 100 normal records, the Q R S duration ranged from 0·03 to 0·11 sec. The minimum value of 0·03 sec. was found in Leads I and III, while the maximum value of 0·11 sec. was observed only twice, once in Lead II and once in Lead III. Values of 0·10 sec. were observed in all the three leads.

The average value for Q R S duration, taking all leads into consideration, was 0·066 sec. Average figures for the three leads, separately, were: Lead I = 0·064 sec.; Lead II = 0·068 sec.; Lead III = 0·066 sec.

For the Q R S duration in children, the majority of investigators have obtained values between 0·06 and 0·072 sec.

TABLE IX

Duration of the Q R S Complex in Adults

Authority	Mean or Average	Range	Maximum Duration of Q R S
Lewis and Gilder[8] 1912	..	..	0·10 sec.
Pardee[13] (1933)	..	0·06 to 0·10	0·10 sec.
Jensen, Smith and Cartwright[6] (1932)	..	0·06 to 0·08	..
McGinn and Paul White[10] (1933) ..	0·078	..	..
	0·083 (Males)	..	..
	0·072 (Females)		
Shipley and Hallaran[15] (1936) ..	0·087 (Males)	..	..
	0·085 (Females)	..	..
Hoskin and Jonescu[3] (1940) .. .	0·06 sec.	0·03 to 0·09	0·09 sec.

TABLE X

Duration of the Q R S Complex in Childhood

Authority	Mean Value	Range
Seham[14] (1921)	0·05 sec. (1 to 5 years)	..
	0·07 ,, (over 5 years).	..
Lincoln and Nicolson[9] (1928) ..	0·06 ,,	..
McGinn and White[10] (1933) ..	0·072 ,.	..
Present series	0·066 ,,	0·03 sec. to 0·10* sec.

*Values over 0·10 sec. for the Q R S duration were found on two occasions only ; a duration of 0·11 sec. was found once in Lead II and once in Lead III.

It is possible that the shorter duration of Q R S in children may be due to a shorter conduction system and a smaller ventricular mass, than in adults.

(For Q R S duration in my 100 cases, see Table XI).

TABLE XI

Duration of Q R S Complex in 100 Cases (Percentages)

Duration of Q R S (in seconds)	Lead I	Lead II	Lead III
0·03	3	0	1
0 04	10	6	6
0·05	17	15	14
0·06	25	25	29
0·07	17	21	19
0·08	18	22	23
0·09	2	6	—
0·10	8	4	7
0·11	0	1	1

Duration of Q R S in the Lead of largest measurement.—Since the Q R S complex represents the time taken by the contraction to spread through the ventricular musculature, the largest value of Q R S duration obtained in a given record, in whatever lead that may happen to be, is the correct measurement to adopt.

The hundred records in my series were investigated from this point of view. Maximum values of Q R S duration were observed 41 times in Lead I, 56 times in Lead II and 55 times in Lead III. In 12 records, the Q R S duration was constant in all the three leads. Maximum values for Q R S duration were shared by two leads on 28 occasions: 9 times in Leads I and II, 16 times in Leads II and III and 3 times in Leads I and III. Values for maximum Q R S duration ranged from 0·04 sec. to 0·11 sec. in the 100 records, with a mean value of 0·074 sec. (see Table XII, for further details).

TABLE XII

Duration of Q R S in the Lead of Largest Measurement (100 records)

Range of Q R S Duration (in seconds)	Lead I	Lead II	Lead III	For all Leads
0·04 to 0·06	11	21	20	28
0·07 to 0·08	22	26	26	54
0·09 to 0·10	8	8	8	16
0·11 to 0·12	0	1	1	2

The Q Deflection or Wave

This is the initial ventricular deflection. The Q Wave is of great import-ance in electrocardiography, as variations and abnormalities of this wave frequently portend serious disease of the heart or coronary arteries.

Incidence of the Q Wave.—The normal Q Wave has been extensively studied by Kossman, Shearer and Texon[5] (1938); they found the presence of a Q Wave in Lead I in 40% of records, in Lead II in 60% and in Lead III in 60%: a Q Wave was observed in Leads II and III in 40% of cases.

In my series of 100 normal school-boys, a Q Wave was present in Lead I in 64% of cases, in Lead II in 66% of cases and in Lead III in 81% of cases. In other words, in a series of 100 cases, the Q deflection was absent in Lead I on 36 occasions, in Lead II on 34 occasions and in Lead III on 19 occasions. A Q Wave was observed in all the three leads in 31 records. In 54 records, a Q Wave was observed in two leads, 14 times in Leads I and II, 20 times in Leads I and III and 20 times in Leads II and III. In 9 records, the Q Wave was confined to one lead only, once to Lead I, 5 times to Lead II and 3 times to Lead III. In 6 records (6%), there was no evidence of a Q Wave in any of the leads.

Amplitude of Q Wave.—The height of the Q Wave varied from 0·0 to 6·0 mm. in the 100 records of my series. The mean value was 0·57 mm.

(For further details about Q Wave amplitude, see Tables XIII and XIV).

TABLE XIII

Q Wave Amplitude in 100 Cases (Range and Mean Values)

	Lead I (in mm.)	Lead II (in mm.)	Lead III (in mm.)	For all Leads (in mm.)
1. Range	0·0 to 4·0	0·0 to 2·1	0·0 to 5·0	0·0 to 6·0
2. Average Value ..	0·465	0·380	0·860	0·570

TABLE XIV

Q Wave Amplitude in 100 Cases (Percentages)

Q Wave Amplitude (Range) (in mm.)	Lead I	Lead II	Lead III
0·0 to 0·5	32	34	28
0·6 to 1·0	13	22	13
1·1 to 1·5	16	6	13
1·6 to 2·0	2	3	11
2·1 to 2·5	0	1	4
2·6 to 3·0	0	0	1
3·1 to 3·5	0	0	0
3·6 to 4·0	1	0	0
4·1 to 6·0	0	0	1
Absence of Q Wave	36	34	19

The R Deflection or Wave

This is an upright ventricular deflection which starts immediately after the Q Wave and ends in the S Wave. The R deflection is usually the most conspicuous of all deflections. The amplitude of R is measured from the iso-electric line to the summit of the R Wave. In the great majority of cases, the R Wave has two limbs, an upstroke and a downstroke. In a few cases, especially in Lead III, the R deflection displays anomalous forms; it may be vibratory, M shaped or W shaped.

Amplitude of R.—The amplitude or height of the R Wave varies enormously. In my series of 100 cases the height of R, in the three leads, ranged from a minimum of 0·4 mm. in Lead III to a maximum of 20·2 mm. in Lead II. The mean value for all leads was 7·06 mm. The R Wave amplitude values were higher in Lead II than in the other leads, in the great majority of cases. In 76% of records, largest amplitudes of R Wave observed in Lead II, in 20% of records in Lead I and in 4% of records in Lead III.

In Tables XV and XVI are recorded my analysis of the amplitude of R in 100 normal records.

TABLE XV

Size of R Wave in 100 Cases (Range and Mean Values)

	For All Leads (in mm.)	Lead I (in mm.)	Lead II (in mm.)	Lead III (in mm.)
1. Range	0·4 to 20·2	1·2 to 16·0	2·5 to 20·2	0·4 to 18·0
2. Mean Value	7·06	6·6	9·2	5·47

TABLE XVI

Size of R Wave in 100 Cases (Percentages)

Size of R Wave (Range) (in mm.)	Lead I	Lead II	Lead III
0·0 to 5	34	6	54
5 to 10	53	56	34
10 to 15	12	33	6
15 to 20	1	3	2
20 to 25	0	2	0

Out of 100 records in my series, there were 19 with the R Waves in all leads under 7 mm. in height. The maximum deflection of R in these 19 cases measured from 5·0 to 6·9 mm.; there was no evidence of heart disease in any of these cases. There was not a single case with the maximum R deflection less than 5 mm. in height.

The height of the R deflection in the lead of largest measurement, according to Pardee,[13] may vary from 7 to 17 mm., except in cases where one lead is diphasic or iso-electric with the amplitude in the other two leads about equal; in such cases, the normal range for the R deflection is 6 to 15 mm. (Pardee).

In Lewis's[7] series of 52 normals, the largest wave of the Q R S complex varied in amplitude from 5·5 mm. to 16·5 mm. with an average of 11 mm. According to Paul White,[17] the normal limits for the R Wave height are 5 mm. and 35 mm. in adults and 5 mm. and 10 mm. in children.

The lower limit of normal for the R Wave has been fixed differently by different authors. Both Pardee[13] and Steuer[16] fix the lower limit of R as 7 mm.; Steuer found severe myocardial lesions in each of fifty cases with

low voltage curves. Paul White[17] fixes the lower limit for the R Wave as 5 mm.; this view has been upheld of late by Hoskin and Jonescu.[3]

In my series of cases, there were 19 records, otherwise normal. with R Waves under 7 mm. in amplitude. These cases were quite free of symptoms and signs of heart disease. There was not a single record with the maximum amplitude of R under 5 mm. On the basis of these figures one is inclined to the suggestion of Paul White to fix the lower limit of R at 5 mm.

The S Deflection or Wave

This deflection immediately follows the R deflection and forms the third or last deflection of the Q R S complex. It is practically always directed in a downward direction. Variations and anomalies of the S Wave are not only less common than those of the Q or R Waves but also carry much less significance. The S Wave gives very little information about the state of the heart-muscle or the coronary circulation; it does, however, help us in the determination of the electrical axis deviation.

Observations on the S Wave.—An S Wave was present in the great majority of my records; an S Wave was observed in Lead I in 85% of records, in Lead II in 85% and in Lead III in 67%. The corresponding values for the Q Wave were 64%, 66% and 81% respectively. It is therefore apparent that while the Q Wave is more frequently observed in Lead III than in either of the other leads, the reverse holds good in the case of the S Wave.

The amplitude or size of the S Wave ranged from 0.0 mm. to 10.1 mm. in the 100 cases, the mean value for all cases being 1.44 mm. The corresponding values for the Q Wave were: range $= 0.0$ to 6.0 mm.; average figure $= 0.57$ mm. The S Wave is therefore a more prominent (though less important) deflection than Q.

In Lead I, the size of the S Wave ranged from 0.0 to 6.0 mm., in Lead II from 0.0 to 10.1 mm. and in Lead III from 0.0 to 8.5 mm. The average values of S for the three leads were: Lead I $= 1.55$ mm.; Lead II $= 1.50$ mm.; Lead III $= 1.26$ mm.

In three records there was a complete absence of the S Wave; S_1 and S_2 were absent in one record; S_2 and S_3 were absent in 7 records; S_1 and S_3 were absent in 2 records.

An analysis of the S Wave is reproduced in Table XVII.

TABLE XVII

Size of S Wave in 100 Records (Percentages)

Size of S Wave (Range) (in mm.)	Lead I	Lead II	Lead III
0 to 2	69	71	85
2 to 4	24	26	7
4 to 6	7	2	4
6 to 8	0	0	3
8 to 10	0	0	1
10 to 12	0	1	0

The amplitude of S in Lead I never exceeded 6 mm.; in Lead II it was over 6 mm. in one record and in Lead III values over 6 mm. were observed in 5 records.

Notching and Slurring of Q R S Complex

| Notching of Downstroke of R Deflection | " Basal Slurring " of Upstroke of R Deflection | " Apical Slurring " of Upstroke of R Deflection |

FIG. 2

Common Varieties of Slurring and Notching of the R Deflection

Notching.—According to Pardee,[13] notching of the Q R S complex may occur even normally in some records, especially in Lead III; occasionally, notching occurs as a normal variation in Lead I but not in Lead II.

In my series of 100 cases, notching of R was not observed in Leads I or II in any of the records. Notching of the R Wave in Lead III was observed 16 times. In other words, 16% of normal records showed notched Q R S complexes in Lead III. The majority of R_3 deflections with notching were " vibratory " in form; the rest were R Waves of small amplitude (from 2·0 to 4·5 mm. in amplitude) with but one exception; in one case, notching was observed in an R deflection (in Lead III) with an amplitude

of 7·5 mm. Definite notching of the S Wave was observed in one record only, in Lead III. Notching of Q R S is, therefore, not a feature seen in normal records, except in Lead III with small or vibratory ventricular complexes.

Slurring or thickening of the limbs of the R or S Waves is frequently observed in normal records. There are, however, two distinct forms of slurring which need to be differentiated, *viz.*, " basal slurring " and " apical slurring "; sometimes, these two forms of slurring are associated in one wave. Basal slurring is much more frequent in normal records and unlike apical slurring is of little or no significance. Apical slurring, on the other hand, is frequently indicative of myocardial pathology.

By *Basal slurring* of R or S, is meant a thickening or splaying out of the limbs of R or S at its junction with the iso-electric or base-line. It is common enough in normal records not to be considered pathological. For instance, basal slurring of the R Wave was observed in 29% of my records. In two records, there was basal slurring of R in all the three leads. In 12 records, it was observed in two leads, 3 times in Leads I and II, 3 times in Leads I and III and 6 times in Leads II and III. In 18 records, basal slurring of R was confined to one lead only; 4 times to Lead I, 6 times to Lead II and 8 times to Lead III.

By " Apical Slurring " of R is meant a thickening or splaying out of one or both limbs of the R Wave, not continuous with or adjacent to the base-line; the thickening is usually nearer the summit than the base of the R Wave (Fig. 2). This type of slurring is said to carry the same significance as notching of the R Wave. According to Pardee,[13] apical slurring can only be considered normal if it is confined to one lead only and if the Q R S complex of that lead has a relatively small excursion. Apical slurring occurring in two or more leads is always pathological (Pardee).

In my series of 100 cases, " apical slurring " of R was observed in as many as 11 records (11%); in all these records, the slurring was confined to one lead only; it occurred on four occasions in Lead I, once in Lead II and 6 times in Lead III.

R Waves with " apical slurring " were of small amplitude in all cases with but one exception; in this case, apical slurring was observed in R Waves with an amplitude of 7·5 mm. The amplitude of R in the other ten records with apical slurring varied from 1·2 to 5·0 mm. with an average of 3·9 mm.

In 4 records, there was combined notching and slurring of the R Waves.

Slurring seems to affect the ascending and descending limbs (upstroke and downstroke) of R and S about equally, there being no special predilection for either limb. For instance, apical slurring of R was observed 6 times in the upstroke and 5 times in the downstroke of that wave.

The S–T Interval

The S–T interval is measured from the end of S to the beginning of the T Wave. It is of the utmost importance in electrocardiography, since variations of this interval are frequently observed in pathological conditions, especially of the coronary arteries and in digitalis intoxication. The S–T interval may be iso-electric or displaced above or below the base-line of the record.

Various forms of S–T interval have been described by Pardee;[13] some of these occur under normal circumstances; others are confined to diseased conditions of the heart.

Duration of the S–T Interval.—The measurement of this interval may be extremely difficult in some cases; especially when the onset of the T Wave is not clearly defined. In a few records, there is an absence of the S–T interval, the upstroke of T taking origin directly from the S Wave.

Hoskin and Jonescu[3] give the average values of the S–T interval for the three leads as follows: For Lead I, 0·11 sec.; for Lead II, 0·11 sec.; for Lead III, 0·13 sec. They found a complete absence of the S–T interval in a large number of their records; their maximum value of 0·24 sec. for the S–T interval was observed in Lead III.

In my series of 100 cases, the duration of the S–T interval in the three leads, ranged from 0·02 to 0·16 sec. The minimum value of 0·02 sec. was only observed once in Lead III; the maximum value of 0·16 sec. was observed once in Lead II and 6 times in Lead III. The average value of S–T interval for the three leads was 0·083 sec.

In Lead I, the duration of the S–T interval ranged from 0·04 to 0·14 sec. with a mean value of 0·08 sec.; in Lead II, from 0·04 to 0·16 sec. with a mean of 0·081 sec.; in Lead III, from 0·02 to 0·16 sec., with a mean of 0·092 sec. It will be observed that the figures for S–T interval duration are definitely lower in my series of cases than in that of Hoskin and Jonescu.[3] An analysis of the S–T interval is reproduced in Table XVIII.

TABLE XVIII

Duration of S–T Interval in 100 Cases* (Percentages)

Duration of S–T Interval (Range) (in seconds)	Lead I	Lead II	Lead III
0·02 to 0·04	0	0	:
0·04 to 0·06	13	15	–
0·06 to 0·08	23	25	:2
0·08 to 0·10	31	21	24
0·10 to 0·12	14	17	15
0·12 to 0·14	9	7	13
0·14 to 0·16	2	4	6
0·16 and over	0	1	6

* The duration of the S–T interval could not be measured in Lead I in 4 records, in Lead II in 3 records and in Lead III in 7 records.

In one record, the duration of S–T was identical in the three leads, the value being 0·06 sec. In 31% of records, maximum values for the duration of the S–T interval were observed in Lead I. Of these 31 records, 6 showed similar duration for S–T in Lead III and 3 in Lead II. In 30% of records, maximum values occurred in Lead II; of these, 10 records showed similar values in Lead III and 3 in Lead I. In 57% of records, maximum values were observed in Lead III; in 6 of these, similar values were found in Lead I and in 10, similar values in Lead II. From these figures, it is obvious that maximum values for the duration of the S–T are most frequently observed in Lead III.

Relation of the S–T Segment to the base-line.—According to Shipley and Hallaran,[15] the level of the S–T segment is influenced by both ventricular and auricular events. The so-called " auricular T Wave " is superimposed, to some extent, on the S–T segment.

Deviation of the S–T segment from the P–R level was measured in the 100 records of the present series. In Lead I, the S–T segment was at the level of the P–R segment in 42%, raised in 14% and depressed in 44% of cases. Depression of the S–T segment was much more frequently observed than elevation, in Lead I (ratio of 3 : 1); this finding is diametrically opposed to that of Hoskin and Jonescu[3] who investigated the records of fifty normal adults. In Lead II, the S–T segment was raised in 17%, depressed in 52%

and at the level of the P–R in 31%; in other words, depression of the S–T segment was three times as common as elevation. In Hoskin and Jonescu's[3] series, elevation of the S–T segment was much more common than depression, in Lead II. In Lead III, the S–T segment was raised in 23%, depressed in 24% and at the level of the P–R in 53%. Hoskin and Jonescu[3] found the S–T segment in Lead III more often depressed than raised.

Deviation of the S–T segment from the level of the P–R seldom exceeds 1 mm. In my series, upward deviation of S–T ranged from 0·1 to 1·2 mm. The maximum figure of 1·2 mm. was attained only once in Lead II. Upward deviation of over 1 mm. was found in only 6 records. Depression of the S–T segment ranged from 0·1 to 1·8 mm. in the 100 cases. The maximum value of 1·8 was only attained once, in Lead I. Values over 1 mm. were found in only 7 records (1·8 mm. in one record, 1·4 mm. in 2 records, 1·3 mm. in 1 record, 1·2 mm. in 2 records and 1·1 mm. in 1 record). In 10 records, the S–T segment was found depressed in all the three leads. Elevation of the S–T segment in all three leads was observed only once.

In 8 records, there was elevation of S–T in one lead, depression of S–T in one lead and iso-electric S–T in the remaining lead. In 6 records, S–T was raised in two leads and depressed in the remaining lead. In 10 records, S–T was depressed in two leads and raised in the remaining lead. 6 records showed raised S–T in two leads with an iso-electric S–T in the remaining lead. 19 records showed depressed S–T in two leads with an iso-electric S–T in the remaining lead. In 26 records, deviation of S–T was confined to one lead only; of these, 7 showed elevation and 19 showed depression of the S–T segment.

In 13 records, the S–T segment was iso-electric in all leads.

The S–T Duration

This is measured from the end of the S Wave to the end of the T Wave. It differs from the measurement known as the " S–T interval " in including the T Wave. S–T duration = S–T interval − T Wave duration.

In the series reported by Hoskin and Jonescu,[3] the S–T duration ranged from 0·16 to 0·36 sec. in the fifty cases. The average values for Leads I, II and III were 0·28 sec., 0·27 sec. and 0·25 sec. respectively.

In my series of 100 cases, the S–T duration ranged from 0·14 to 0·36 sec., in the three leads. The minimum value of 0·14 sec. was found only once in Lead II; the maximum value of 0·36 sec. was noted twice in Lead II and once in Lead I. The mean value for all leads was 0·245 sec.

In Lead I, S–T duration ranged from 0·16 sec. to 0·36 sec. with an average of 0·24 sec.; in Lead II, it ranged from 0·14 to 0·36 sec. with an average of 0·25 sec.; in Lead III, the range was 0·15 sec. to 0·33 sec. and the average, 0·245 sec.

An analysis of S–T duration is reproduced in Table XIX.

TABLE XIX

The S–T Duration in 100 *Records* (Percentages)*

S–T Duration (Range) (in seconds)	Lead I	Lead II	Lead III
0·13 to 0·15	0	1	1
0·16 to 0·20	20	15	18
0·21 to 0·25	38	40	29
0·26 to 0·30	36	34	30
0·31 to 0·35	4	7	4
0·36 to 0·37	1	3	0

* The S–T duration could not be measured in 1 record in Lead I and in 18 records in Lead III, owing to the iso-electric character of the T Waves.

The T Wave or the Second Ventricular Deflection

The T Wave forms the last of the ventricular deflections. Variations of the T Wave have been exhaustively studied by cardiologists, as they frequently portend serious myocardial and coronary diseases.

Direction of the T Wave.—In normal records, T Waves in Leads I and II are invariably upright or directed upwards. This view is held by the great majority of cardiologists. It does not however apply to the T Wave in Lead III. According to Pardee,[13] the T Wave in Lead III is inverted or negative in about one-fifth of normal records, diphasic in somewhat less than one-fifth of all records and upright or positive in two-thirds of normal records. In the series reported by Hoskin and Jonescu,[3] inversion of T Wave in Lead III was noted in 46% of records.

In my series of 100 records from normal school-children, the T Waves in Leads I and II were upright in all cases; T_3 was upright or positive in 34% of records, inverted or negative in 46%, diphasic or bi-directional in 4% and flat or iso-electric in 16%. In Lead III, therefore, the T Wave was more often inverted than upright.

Form of the T Wave

Upright forms (Pointed and Rounded)   Diphasic forms

Iso-electric form   Inverted forms (Pointed and Rounded)
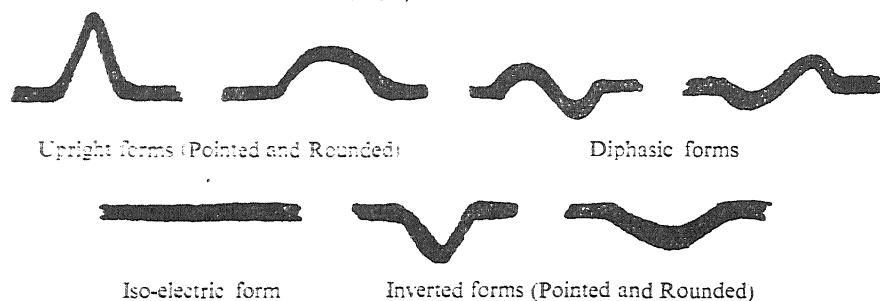
FIG. 3

Different Forms of T Wave (Diagrammatic)

The form of the T Wave is almost as variable as that of the P Wave. Pointed upright, rounded upright, inverted, diphasic and iso-electric forms of T Wave are commonly encountered in normal records in Lead III. T Waves can be separated into two broad groups, according to contour, *viz.*, the " pointed form " of T and the " rounded form " of T. This distinction also holds good in the case of inverted T Waves.

In my sereis of 100 cases, the " pointed " or " peaked " form of T Wave was about twice as common as the " rounded form " (ratio of 9:5) taking all leads into consideration. In Lead I, the T Wave was " pointed " in 74 records, " rounded " in 20 and of the " mixed variety " (rounded and pointed) in 6 records. In Lead II, the T Wave was " pointed " in 67, " rounded " in 30 and " mixed " in 3 records. In Lead III, the T Wave was " pointed " in 33 and " rounded " in 46 records; in 16 records, it was iso-electric and in 5 records, it was diphasic. The proportions of pointed: rounded forms of T in the three leads were as follows: In Lead I a ratio of 3·7:1; in Lead II, 2·2:1; in Lead III, 1:1·4.

In 24 records, T was " pointed " or " peaked " in all leads; in 9 records, T was " rounded " in all leads.

Amplitude of T Waves in General.—In my series of records, the amplitude or size of the T Wave in Lead I ranged from 0·4 to 9·0 mm., in Lead II from 0·5 to 9·5 mm. and in Lead III from −4·1 to 5·5 mm. Average values for T Wave amplitude were: Lead I = 3·45 mm.; Lead II = 3·40 mm.; Lead III = −0·05 mm.

The mean value of T, for all leads, was 2·25 mm. An analysis of T Wave amplitude is reproduced in Table XX.

TABLE XX

Size of T Wave in 100 Cases (Percentages)

Size of T Wave (Range) (in mm.)	Lead I	Lead II	Lead III
−6·0 to −4·0	0	0	1
−4·0 to −2·0	0	0	7
−2·0 to 0·0	0	0	41
0·0 to 2·0	19	25	25
2·0 to 4·0	47	44	13
4·0 to 6·0	24	18	1
6·0 to 8·0	7	7	0
8·0 to 10·0	3	6	0

Amplitude of T Wave in the Lead of Largest Measurement.—According to Pardee,[13] the height of the T Wave in the largest lead varies from 1·5 to 5·0 mm. except when one lead of the record is diphasic or iso-electric; in that case, the normal value for T becomes 1·3 to 4·5 mm. The maximum value of T in Pardee's series was 5·5 mm.; he observed this value on two occasions. In Lewis'[7] series of normals, the maximum value of T was also 5·5 mm.; he observed this value only once. In Pardee's[13] opinion, the amplitude of T in the lead of largest measurement is seldom under 2 mm.; he found a T Wave amplitude of 1·5 mm., only once in his series of normals. Though Pardee considers values under 1·5 mm. (for T in the lead of maximum measurement) definitely abnormal, Lewis[7] observed as many as five normal cases with the value of T as low as 1·0 mm.

In my series of 100 cases, the value of T in the lead of largest measurement, ranged from 1·2 to 9·5 mm. Values under 2·0 mm. were observed in 5 records only (5%); the T values in these records were 1·5 mm., 1·4 mm., 1·8 mm., 1·2 mm. and 1·5 mm. Values over 5 mm. were observed quite frequently, in as many as 23% of records.

Maximum amplitudes of T were observed in Lead I in 55% of cases, in Lead II in 44% and in Lead III in 1% only. Maximum values of T are therefore hardly ever found in Lead III.

While the average height of T in the lead of largest excursion, in the combined series of Lewis and Pardee, was 3 mm. it was 4 mm. in the present

series. This difference in average value is probably explained by the difference in age of the subjects employed in the two series of cases. While Lewis and Pardee both worked on normal adults, the present investigation was restricted to the age-group of 5 to 15 years. Well-defined and large T Waves have been observed in childhood by various workers (Burnett and Taylor[2]). According to Burnett and Taylor, T_1 and T_2 are much larger in children than in adults: T_3 is about equal in the two cases.

(For further details please see Table XXI).

TABLE XXI

*Amplitude of T in the Lead of Largest Excursion (**Case Numbers**)*

Amplitude of T (Range) (in mm.)	Lead I	Lead II	Lead III
—2·0 to 0·0	0	0	1
0·0 to 2·0	7	3	0
2·0 to 4·0	30	17	0
4·0 to 6·0	17	12	0
6·0 to 8·0	1	6	0
8·0 to 10·0	0	6	0
10·0 to 12·0	0	0	0
TOTAL	55	44	1

Duration of T Wave.—The duration of the T Wave is measured from the beginning of the upstroke to the end of the downstroke of the T Wave, when the latter wave is upright. When the T Wave is inverted, as it frequently is in Lead III, the duration of T is measured from the beginning of the downstroke to the end of the upstroke of T. T Wave duration cannot be measured in records with iso-electric or flat T Waves.

In my series of 100 cases, the duration of T was very variable; it ranged from 0·08 to 0·26 sec., with an average value of 0·160 sec. for all leads. The duration of T in Lead I ranged from 0·10 to 0·26 sec., in Lead II from 0·10 to 0·24 sec. and in Lead III from 0·08 to 0·21 sec. The average values for the three leads were: Lead I = 0·175 sec.; Lead II = 0·172 sec.; Lead III = 0·138 sec.

The corresponding values for T in Leads I, II and III in the series of Hoskin and Jonescu,[3] were 0·16 sec. and 0·12 sec. respectively.

The minimum value of 0·08 sec. for T Wave duration in my series was noted in 5 records, in Lead III only. The maximum value of 0·26 sec. was noted only once, in Lead I.

As in the series observed by Hoskin and Jonescu[*] no relationship was observed between the height and the duration of the T Waves.

An analysis of T Wave duration is reproduced in Table XXII.

<div align="center">

TABLE XXII

Duration of T Wave in 100 Records* (Percentages)

</div>

Duration of T (Range) (in seconds)	Lead I	Lead II	Lead III
0·08 to 0·10	2	1	15
0·10 to 0·12	6	7	13
0·12 to 0·14	17	10	19
0·14 to 0·16	17	27	17
0·16 to 0·18	26	23	6
0·18 to 0·20	17	23	6
0·20 to 0·22	5	5	1
0·22 to 0·24	8	4	1
0·24 to 0·26	2	0	0

* In 16 records, the duration of T in Lead III could not be measured owing to the iso-electric character of the T Wave.

Q–T Duration or Duration of the Ventricular Complex.—In Pardee's[13] opinion, it is incorrect to measure the duration of the ventricular complex from the end of Q R S to the end of T, since ventricular activity starts at the onset and not at the end of the Q R S complex.

The correct measurement of Q–T or duration of the ventricular complex is from the beginning of Q R S to the end of the T Wave. The Q–T duration should be measured in that lead of the electrocardiogram, in which it is longest. This was done in each of the 100 records of my series. Maximum values of Q–T duration were observed in Lead I on 32 occasions, in Lead II on 57 occasions and in Lead III on 20 occasions. Maximum values of Q–T were shared by two leads in 10 cases; 8 times in Leads I and II, once in Leads II and III and once in Leads I and III. The duration of Q–T ranged

from 0·20 to 0·46 sec. in the 100 records, the average value being 0·345 sec (see Table XXIII.)

TABLE XXIII

The Q-T Duration (Maximum) in 100 Records

Lead	Number of records	Average value (in seconds)	Lowest value (in seconds)	Highest value (in seconds)
Lead I	32	- 0·345	0·22	0·42
Lead II	57	0·340	0·22	0·46
Lead III	20	0·350	0·20	0·40
For all Leads ..	100	0·345	0·20	0·46

The Q-T Duration in Relation to the Heart-rate.—There appears to be some relationship between the duration of the ventricular complex and the rate of the heart. Such a relationship was observed in 26 normal cases and tabulated by Pardee.[13]

All the records in my series were studied from this point of view. A definite relation was observed between the duration of Q–T and the rate of the heart. There was a gradual diminuition in the duration of Q–T with increasing rate of the heart.

(See Table XXIV and Figs. 4 and 5).

TABLE XXIV

Q-T Duration in Relation to the Heart-rate

Rate of Heart (per min.)	Number of cases	Average value (in seconds)	Highest value (in seconds)	Lowest value (in seconds)
60 to 70	5	0·374	0·41	0·34
71 to 80	4	0·370	0·40	0·33
81 to 90	18	0·350	0·42	0·28
91 to 100	32	0·335	0·46	0·22
101 to 110	20	0·314	0·36	0·20
111 to 120	8	0·295	0·32	0·25
121 to 130	3	0·294	0·33	0·26
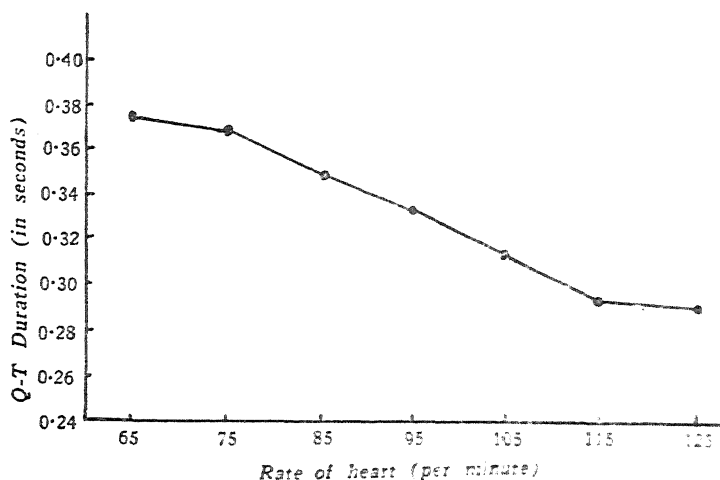
FIG. 4

Average values of Q-T duration for different rates of the heart
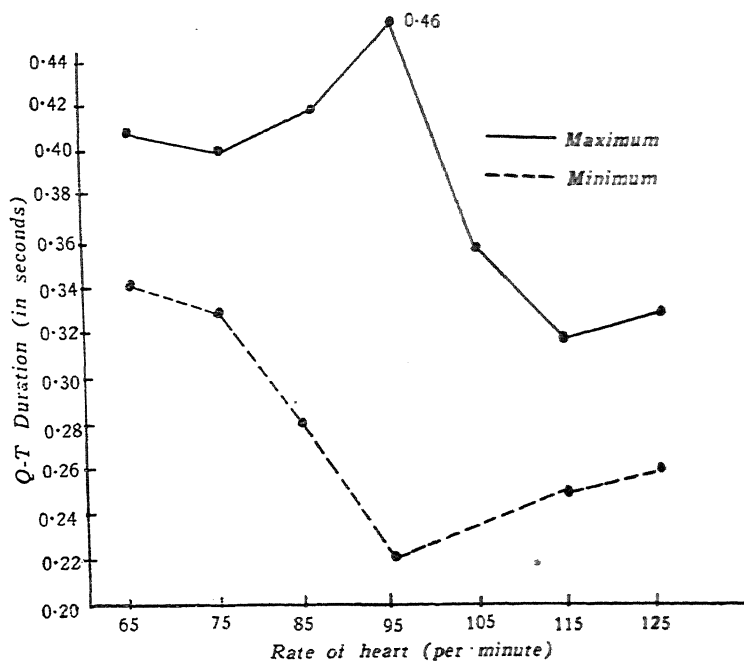
FIG. 5

Maximum and minimum values of Q–T duration for different rates of the heart

The U Wave

The U Wave or " the sixth wave of the electrocardiogram " was first noted by Einthoven in 1906. He observed it in about half of his records. Lewis and Gilder[8] (1912) who made a study of this wave in normal adults, found it in about 90% of adults and most frequently in Lead II. They described two forms of U Waves: (1) a pointed or peaked form, immediately after the T Wave, and (2) a shallow, rounded form of U of long duration, occupying most of the T–P segment.

The cause of the U Wave is not clear. Many different hypothesis have been put forward to explain its mode of origin. Whether it is cardiac in origin (Hoffman, 1914; Kahn, 1914), aortic in origin (Hering, 1913) or arises in the great vessels (Pardee, 1924) is open to question.

The normal U Wave has been observed at all ages from 16 to 75 and in both sexes (Papp,[12] 1940). The incidence of this Wave in childhood has not as yet been determined. Krumbhaar and Jenks[6] (1917), Scham[14] (1921), Lincoln and Nicolson[9] (1928) and Bruce[1] (1931) make no allusion to the U Wave in their papers on electrocardiograms in childhood.

The height of the normal U Wave in the standard leads, varies from 0·10 to 1·0 mm.; duration of the U Wave varies from 0·16 to 0·24 sec. (Papp,[12] 1940).

Various abnormalities of the U Wave have recently been described in diseases of the heart by Nahum and Hoff[11] (1939) and by Papp[2] (1940). Physiological factors are also capable of influencing the form and duration of this wave.

The U Wave was investigated in the 100 records of the present series. U Waves were observed in one or more leads in 50 of the 100 records, giving an incidence rate of 50%. In 5 records, U Waves were observed in all the three leads. U Waves were observed in two leads in 17 records, 5 times in Leads I and II, 10 times in Leads II and III and twice in Leads I and III. In 28 records, the U Wave was confined to one lead only, 21 times in Lead II, 6 times in Lead III and only once in Lead I.

Form of U Wave.—Two main forms of U Wave were encountered: (1) the " pointed " or peaked form, with short duration, and (2) the " rounded form " occupying most of the T–P segment.

In 28 records, U Waves were of the " rounded " variety only ; in 15 records, they were all of the " pointed variety; in 7 records, both pointed and rounded forms were observed. In my series of records, the rounded form of U was about twice as common as the pointed form.

Incidence of U Waves in Different Leads.—U Waves were most frequently encountered in Lead II. They were observed in Lead I in 13 records, in Lead II in 41 records and in Lead III in 23 records. The " pointed " form of U Wave was observed in Lead III 8 times and in Lead I only once (see Table XXV).

TABLE XXV

Incidence of U Waves in the three Standard Leads

		Number of records with U Waves	No. of records with " rounded " U Waves	No. of records with " pointed " U Waves
Lead I	..	13	12	1
Lead II	..	41	25	16
Lead III	..	23	15	8

The *amplitude* of U ranged from 0·1 mm. to 1·5 mm. in the 100 cases, with an average value of 0·54 mm. The average amplitude of the " pointed form " of U was found to be about three times that of the " rounded from " (see Table XXVI).

TABLE XXVI

Amplitude of the U Wave in 100 Records

		Average value (in mm.)	Maximum value (in mm.)	Minimum value (in mm.)
1. U Waves in general	..	0·54	1·5	0·1
2. " Pointed form " of U	..	0·80	1·5	0·2
3. " Rounded form " of U	..	0·29	1·2	0·1

The *duration* of U ranged from 0·05 sec. to 0·24 sec. in the 100 cases, with an average value of 0·13 sec. The average duration of the " rounded form " of U was about twice that of the " pointed form ".

TABLE XXVII

Duration of the U Wave in 100 Records

		Average value (in sec.)	Maximum value (in sec.)	Minimum value (in sec.)
1. U waves in general	..	0·13	0·24	0·05
2. " Pointed form " of U	..	0·095	0·24	0·05
3. " Rounded form " of U		0·15	0·20	0·08

TABLE XXVIII

A Summary of the Main " Waves "of the Electrocardiogram

Wave	Feature investigated	Average value	Maximum value	Minimum value	Measurements in Lead of largest measurement
P Wave ..	Amplitude	0·90 mm.	3·1 mm.	0·0 in Leads I and II. −1·0 in Lead III	
	Duration	0·08 sec.	0·14 sec.	0·04 sec.	
Q R S Complex ..	Duration	0·066 sec.	0·10 sec.	0·03 sec.	Range : 0·04 to 0·11 sec. Average: 0·074 sec.
Q Wave .	Amplitude	0·57 mm.	6·0 mm.	0·0 mm.	
R Wave ..	Amplitude	7·06 mm.	20·2 mm.	0·4 mm.	
S Wave .	Amplitude	1·44 mm.	10·1 mm.	0·0 mm.	
T Wave ..	Amplitude	2·25 mm.	9·5 mm.	0·4 mm. (In Lead III : −4·1)	Range : 1·2 to 9·5 mm. Average : 4 mm.
	Duration	0·160 sec.	0·26 sec.	0·08 sec.	
U Wave ..	Amplitude	0·54 mm.	1·5 mm.	0·1 mm.	
	Duration.	0·13 sec.	0·24 sec.	0·05 sec.	

TABLE XXIX

A Summary of the Main " Intervals " of the Electrocardiogram

Interval	Feature investigated	Average value	Maximum value	Minimum value	Measurements in Lead of largest measurement
P–Q duration .	Duration	0·062 sec.	0·12 sec.	0·01 sec.	
P–R interval .	Duration	0·142 sec.	0·21 sec.	0·07 sec.	Range : 0·09 to 0·21 Average : 0·153 sec.
P–R level ..	Deflection	0·27 mm.	2·0 mm.	0·0 mm.	
S–T interval .	Duration	0·083 sec.	0·16 sec.	0·02 sec.	
S–T level	Deflection	..	1·2 (upward) 1·8 (downward)	0·1 mm. (upward) 0·1 mm. (downward)	
S–T duration ..	Duration	0·245 sec.	0·36 sec.	0·14 sec.	
Q–T duration ..	Duration	0·345 sec.	0·46 sec.	0·20 sec.	

Summary

1. A study is presented of 100 normal electrocardiograms of school-boys, ranging in age from 5 to 15.

2. The various deflections and "intervals" of the normal electrocardiogram are measured and analysed; corresponding figures and values from American and European authors are presented for the sake of comparison.

3. Normal electrocardiographic standards are presented for the use of practitioners in India. The results of the investigation are briefly presented in a tabular form (see Tables XXVIII and XXIX).

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THE CHEMISTRY OF GARLIC (*ALLIUM SATIVUM* L.)

Part I. The Nitrogen Complex

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Introduction

Garlic is a condiment which is widely cultivated in India. It has been used from the earliest times for flavouring soups, salads and sausages. It has medical properties and is administered in cases of all fevers and debilitating conditions; it is reported to possess antiseptic and bactericidal properties.

The purpose of this investigation is mainly to find the nature and distribution of nitrogen in the condiment.

Experimental

I. Preparation of the Material

Garlic was obtained from the local market, the outer dry coat and the root stumps removed, crushed in an end-runner to a fine paste and spread over glass plates. Drying was effected in a current of air, by keeping the glass plates in chambers maintained at 40°. After four days, the dry mass was powdered to pass through a sieve of 40 mesh, and stored in a desiccator over calcium chloride as the material exhibits a marked tendency to absorb moisture from the air. No special precaution was taken to prevent hydrolysis of the reserve proteins during the preparation of the material for study.

The proximate chemical composition was found to be as follows:

Table I

Chemical Composition of the Dry Powder Expressed as %

Moisture	Total N.	Ether extraction	Ash	Total sulphur	Total phosphorus	Carbohydrates (by difference)
3·55	1·95	3·39	4·01	0·56	0·41	71·86

II. Nitrogen, Sulphur and Phosphorus Partition of Ether Extract

20 gm. of the material was twice extracted with ether (200 ml. each time), for 24 hours and sulphur, phosphorus and nitrogen determined. Sulphur was determined by the method of Masters (1939) and phosphorus by that of Fiske and Subbarow (1925).

TABLE II

Nitrogen, Sulphur and Phosphorus in Ether Extract
(Expressed as percentage of the extract)

Nitrogen	Sulphur	Phosphorus
0·066	0·072	0·016

III. Nitrogen Partition in different Protein Extractants

The ether extracted material was successively extracted with water, 4% saline, 70% alcohol and 0·1% sodium hydroxide.

Precipitation with trichloroacetic acid was used for distinguishing between protein and non-protein nitrogen in the extract.

TABLE III

Nitrogen Partition in Protein Solvents

Solvent	Total N in the extract as % of the total N in the material	Non-protein N as % of total N of the extract
Water	42·2	74·6
4% saline	25·2	69·2
70% alcohol .. .	8·5	90·2
0·1% sodium hydroxide ..	5·3	65·7

IV. Determination of Non-protein Nitrogen on the Material

In this and all succeeding experiments, the powder which has been previously extracted with ether was used. The method of Ayres and Lee (1936) and Mezincesow and Szabo (1936), using respectively tungstic and trichloroacetic acids as protein precipitants, which have been successfully used with animal tissues were adopted. (Compare Narasimhamurthy and Ranganathan, 1937; and Narasimhamurthy, 1938.)

TABLE IV

Non-protein Nitrogen

Method	N. P. N. expressed as % of dry material	N. P. N. expressed as % of total N
Ayres and Lee .. . (Tungstic acid)	1·20	61·01
Mezincesow and Szabo .. (Trichloroacetic acid)	1·24	61·50

V. Aqueous Extract

(a) *Preparation.*—260 g. of the powder was extracted with 5200 ml. of water on a shaker for 6 hours. After filtering through cloth and kieselguhr the limpid yellow liquid was concentrated at 40°, to a small volume, so that 100 ml. contained 1·3 g. of nitrogen.

(b) *Fractionation according to Wastneys and Borsook.*—The water extract was analysed according to the scheme of Wastneys and Borsook (1924) for incomplete protein hydrolysates. Instead of the " sub-peptone " fraction of these authors, which was obtained by precipitation of the filtrate from tannic acid and treatment with alcohol, material precipitable by phosphotungstic acid in the presence of 5% sulphuric acid was determined.

The residual filtrate containing probably simple amino acids (non-basic), and simple polypeptides gave positive reactions with ninhydrin, Folin's phenol reagent, (tyrosine and tryptophane) and Folin's uric acid reagent (cystine).

TABLE V

Nitrogenous Constituents of Aqueous Extract

Form	Nitrogen as % of total N in the extract
Protein	33·35
Metaprotein ..	..
Proteose	..
Peptone	..
Basic	48·36
Residual	18·29

(*c*) *Nitrogen distribution after hydrolysis.*—29 ml. of the aqueous extract was hydrolysed with 5 volumes of concentrated hydrochloric acid and the nitrogen distribution was determined according to the method of Van Slyke as modified by Damodaran to include dicarboxylic acids. The results are given in Table VI.

TABLE VI

Nitrogen Distribution of the Hydrolysed Aqueous Extract

	Per cent. of total N
Amide N	26·88
Humin N	25·12
Dicarboxylic acid N	13·36
Basic non-amino	21·98
Basic amino	1·22
Non-basic non-amino N	10·64
Non-basic amino N	1·08

(*d*) *Independent estimations of amino acid after hydrolysis.*—1. Arginine was estimated in the hydrolysate by the methods of Hunter and Dauphinee (1930), Van Slyke (1911) and Kossel and Gross (1912).

2. The usual method of Folin and Ciocalteau (1927) for the estimation of tyrosine and tryptophane could not be used as the cloudy coloured experimental solutions could not be satisfactorily matched with the standard. Lugg's (1937, 1938) modified method, however, proved quite satisfactory.

3. Cystine was estimated by Lugg's (1932) modification of Folin and Marenzi's method.

TABLE VII

Amino Acids Expressed as % of Total N in the Aqueous Extract

Arginine N:	
Van Slyke	12·89
Enzyme	11·01
Flavianate	10·40
Tyrosine	10·51
Tryptophane	2·55
Cystine	5·45

VI. Non-protein Fraction

(*a*) *Preparation.*—The meal 300 g. was extracted with 4·5 litres of 0·5% acetic acid, by shaking for 6 hours. The combined filtrates were heated on a boiling water-bath to coagulate the proteins and filtered through a bed of kieselguhr. The clear filtrate which gave no precipitate with trichloroacetic acid, was concentrated under reduced pressure to 350 ml. so that 100 ml. contained 1·00 g. of nitrogen.

(*b*) *Nitrogen distribution after hydrolysis.*—50 ml. of the non-protein extract was hydrolysed with 50 ml. of concentrated hydrochloric acid on a water-bath for 24 hours. A partitioning of nitrogen according to Van Slyke as modified by Damodaran was carried out.

TABLE VIII

	Expressed as % of total N in the non-protein fraction
Amide N	64·52
Humin N	5·66
Dicarboxylic acid N	7·28
Basic amino N	7·27
Basic non-amino N	3·55
Non-basic amino N	5·19
Non-basic non-amino N	5·49

(*c*) *Independent determinations of amino acids.*—Arginine, tyrosine, tryptophane and cystine were determined by the methods already indicated.

TABLE IX

Distribution of Amino Acids

	Expressed as % of total N of the non-protein extract
Arginine N :	
Van Slyke	36·26
Hunter and Dauphinee	12·90
Kossel and Gross	12·40
Tyrosine	16·62
Tryptophane	11·37
Cystine	2·37

VII. Determination of Free Cystine in the Meal

(a) 5 g. lots of the powdered meal was extracted four times with 10% trichloroacetic acid (10 ml. each time) by grinding with twice its weight of acid washed sand, and filtered.   The filtrate was made up to volume, and 10 ml. was brought to pH 5·7 and cystine determined by the method of Lugg (1932).

Table X represents the results of triplicate experiments.

TABLE X

Free Cystine in the Meal

Experiment	Cystine as mg. % of the material	Cystine as % of the total N
1	40·0	2·05
2	42·0	2·02
3	40·0	2·05

(b) Cysteine was tested by the method of Fleming (1930) using dimethyl-*p*-phenylenediamine hydrochloride and ferric chloride, which gave a faint blue colour and the quantity is so small that it could not be determined colorimetrically.

VIII. Nitrogen Distribution in the Material

For assessing the nutritive value of garlic, the amino acid make-up of the whole material is of greater importance than that of the individual proteins isolated from it, notwithstanding the fact that during the process of analysis, partial destruction of some of the amino acids takes place in the presence of extraneous material.

10 g. of the dry powder was hydrolysed for seven days at 110–20°, with 200 ml. of 20% hydrochloric acid and the nitrogen distribution determined according to Van Slyke (1911) as modified by Plimmer and Rosedale (1925). Cystine could not be determined on the basic fraction but was done independently by the modified method of Lugg (1932). Arginine was determined on the basic fraction only, whereas tyrosine and tryptophane were estimated independently by Lugg's modification of Folin and Ciocalteu method.

TABLE XI

Nitrogen Distribution in the Material after Hydrolysis

	Expressed as % of total N of the material
Amide N	12·35
Humin N	15·30
Arginine N	23·70
Histidine N	5·15
Lysine N	6·71
Mono-amino N ..	30·50
Non-amino N	6·01

TABLE XII

Independent Determinatinns of Cystine, Tyrosine and Tryptophane

Amino acid	Expressed as % of total N	Expressed as % of total S	Expressed as % of the total material	Cystine N expressed as % of total N
Cystine	7·69	7·14	0·15	1·23
Tyrosine	..	..	0·18	..
Tryptophane ..	..	..	0·11	..

Conclusions

Nearly 60% of the total nitrogen on the dry weight of the material is in the non-protein form. Many foodstufis, vegetables and pulses contain considerable amounts of non-protein nitrogen (McCance, *M. R. C. Reports*), Bhagvat and Sreenivasaya (1935), and Swaminathan (1938), which may exist as simple derivatives of proteins, amino acids and other nitrogenous substances, not precipitated by the common protein precipitants. By exhaustive extraction with water, about 80% of the total nitrogen is solubilised, of which, again 67% is in the non-protein form. This fraction is largely composed of polypeptides and basic amino acids precipitable by phosphotungstic acid; free cystine, tyrosine and tryptophane are detected in the filtrate from the phosphotungstic acid precipitate.

The nitrogen partitioning of the non-protein fraction reveals two important facts: firstly, the amide nitrogen value is exceptionally high (compare values recorded by Bhagvat and Sreenivasaya (1936) and Rau and Ranganathan (1938) for the non-protein extracts from various vegetable sources; secondly, the arginine values obtained by the Van Slyke and the enzyme methods show a marked discrepancy clearly pointing out to the existence of groups which decompose under the influence of the alkali even after the amide nitrogen had been removed.

TABLE XIII

The Amino Acid Composition of Garlic, Pepper, Chillies, Corriander and that of Proteins obtained from Various Cereals Compared

Amino acid	Eleusinin[1] from *Eleusine coracana*	Oryzanin[2] from *Oryzæ sativa*	Nitrogen complex of *Piper nigrum*[3]	Nitrogen complex of corriander[3]	Nitrogen complex of chillies[4]	Nitrogen complex of garlic
Arginine ..	2·60	17·69	1·18	7·71	0·91	23·65
Histidine ..	2·69	5·39	4·40	1·26	0·98	5·15
Cystine ..	?	0·88	1·48	Traces	Nil	..
Lysine ..	0·64	4·90	6·81	3·48	10·99	6·90
Total protein % of the meal ..	7·10	6·44	12·90	14·71	14·59	12·19

[1] and [2] from Winton and Winton (1932).

[3] Narasimhamurthy and Ranganathan (1938).

[4] Narasimhamurthy (1938).

It will be observed from the results tabulated in Table XIII that garlic contains all the amino acids essential for growth and maintenance in fairly good quantities. A comparison of the amino acid make-up of different condiments and cereals reveals that garlic approximates in composition to pepper; further it contains more cystine, histidine and lysine than the proteins of common cereals, notably ragi, which is very deficient in cystine and lysine.

Therefore garlic may have a useful " supplementary value " when consumed in combination with cereal foods.

Summary

1. An investigation of the chemical composition, and the nitrogen partition in the nitrogen complex of the garlic (*Allium sativum*) has been carried out.

2.   Exhaustive extraction with water removes 80% of the total nitrogen of which 67% is in the non-protein form, largely made up of compounds precipitable by phosphotungstic acid in 5% sulphuric acid.

3.   The non-protein nitrogen extract has been fractioned for nitrogen distribution.   Arginine, cystine, tyrosine and tryptophane have been determined independently.   These results point out the existence of some type of compounds decomposing under the influence of alkali with the liberation of ammonia.

4.   Garlic contains appreciable amounts of most of the important amino acids, its richness in lysine and histidine suggests that it may be of value in nutrition.

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THE CHEMISTRY OF GARLIC (*ALLIUM SATIVUM L.*)

Part II. The Phosphorus Distribution

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It has been mentioned in the previous part that Garlic contains 0·41% phosphorus and this element constitutes more than 10% of its ash. The ether extract of garlic contains 0·016% phosphorus. It thus constitutes an important constituent of garlic and merits a more detailed study.

It has been recognised that the availability of phosphorus present in a foodstuff, depends on the form in which it occurs. Thus following Mellanby (1931), a number of workers have shown that phytin phosphorus is largely unavailable to the organism. The results of a study on the phosphorus distribution of garlic is given in this paper.

Material

Garlic was bought from the local market and freed from the outer dry coat and root stumps.

Methods

Acid-soluble Phosphorus

1. *Extraction.*—Extraction was carried out with trichloroacetic acid which coagulated all its proteins. These could then be centrifuged (Needham *et al.*, 1932; Cori and Cori, 1932). A similar technique was used by the Barenscheen and co-workers (1927–29).

(*a*) A preliminary experiment was performed to find the optimum conditions under which the maximum amount of acid-soluble phosphorus is extracted with the minimum amount of hydrolysis of the phosphoric esters.

10 g. lots of the material was ground to a pulp with 2·5 g. acid-washed sand in the cold room. Then 25 ml. of 7·5% trichloroacetic acid was added, ground well and left at 0° with occasional grinding for 30 minutes. The mixture was filtered (cold) at the pump on a hardened filter-paper, and the filtrate after refiltering through No. 42 Whatman filter-paper, was made up to 30 ml. Total phosphorus was determined in one ml. aliquots after

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incineration. The residue was returned to the mortar and the extractions were repeated thrice over. Table I gives the results of this experiment.

TABLE I

Preliminary experiments to find the conditions suitable for the maximum extraction of acid-soluble phosphorus

Extraction No.	1	2	3	4
Phosphorus extracted as % of total P	36·6	2·8	0·4	0·3

On the basis of this experiment, it was decided to carry out three extractions in cold, using 25 ml. of 7·5% trichloroacetic acid for every 10 g. of the material.

(*b*) 40 g. of the material was crushed in an ice-cold glass mortar with 10 g. of acid-washed sand till the mass was reduced to a soft pulp. Extraction was effected with 100 ml. of 7·5% cold trichloroacetic acid for 30 minutes. The extractions were repeated thrice over.

2. *Fractionation.*—The trichloroacetic acid extract will contain the following groups of phosphorus compounds, if they are present in the material: inorganic ortho-phosphate; labile esters such as adenyl pyro-phosphate; resistant esters such as hexose-phosphates, and trios-phosphates; phosphagens such as arginine and creatine phosphates; and phytin.

Phospholipins and phospho-proteins are the main constituents of the "residual-P" fraction which are determined as the difference between acid-soluble phosphorus and the total phosphorus content of the material determined by incineration.

The separation of acid-soluble phosphorus into various fractions was carried out by the barium precipitation method of Needham *et al.* (1937). The extracts were made up to 300 ml. and 2 ml. aliquots being used for the determination of the total P by incineration (Fiske and Subbarow, 1925). 100 ml. of the extract was treated with 3 ml. of 25% barium acetate and rendered just alkaline to phenolphthalein with 40% cold sodium hydroxide. After standing for 30 minutes at 0°, the flocculent precipitate was centrifuged down and washed on the centrifuge with 8 ml. of 1% barium acetate solution. The precipitate was dissolved in 0·5 ml. of normal hydrochloric acid and diluted to 15 ml. The barium was removed by sodium sulphate, filtered, washed and made up to 30 ml. 1·2 ml. in duplicate were used for the determination of barium-precipitable P, 8 ml.

aliquots for total inorganic phosphate, and further 8 ml. aliquots for hydrolysis in the boiling water-bath with normal hydrochloric acid for 7 minutes giving the value for the pre-formed inorganic ortho-phosphate + the labile ester fraction. The labile ester fraction is then the difference between the last estimate and the inorganic ortho-phosphate estimate previously determined. The " resistant ester P " content can be calculated as the difference between the total barium-precipitable P and the value for P obtained on the aliquot after 7 minutes hydrolysis.

The centrifugate from the barium precipitation was made up to 150 ml. and barium was removed by sodium sulphate from 80 ml. of the centrifugate, and then made up to 100 ml. From this 5 ml. aliquots were used for the determination of the total barium-soluble P. 10 ml. aliquots were kept for 30 minutes at the room temperature (26°) with the molybdate reagent. No phosphorus was liberated and hence phosphagen-phosphorus is absent. Another batch of 10 ml. aliquots were subjected to mild hydrolysis for 7 minutes. The difference between the mild hydrolysis value and the total barium-soluble P gives the amount for the barium-soluble resistant phosphorus. Necessary corrections for the changes in volume were effected.

(3) *Total phosphorus of the material.*—This was determined by incineration of 50 mg. of the material.

4. *Phytin.*—Many workers have determined the phytin phosphorus of cereals by the modification of Hubner and Stradler's (1914) original method. This depends on the extraction of the phytic aicd from the finely ground material by means of hydrochloric acid, and titration of the extract with ferric chloride in the presence of ammonium thiocyanate. The phytin was precipitated as the ferric salt and the end point is indicated by the appearance of a red colour. Numerous workers have experienced difficulty in determining the end point, and modifications have been introduced (Rather, 1917; Averill and King, 1926; Harris and Moscher, 1934). The method of McCance and Widdowson (1935) which precipitates the phytin as the ferric salt and determines phosphorus on the ferric phytate, was employed in this investigation.

5 g. of the powder prepared as in Part I, pp. 268–76, was employed for the determination of phytin. A correction was made for moisture.* The results are presented in Table II.

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* Moisture was determined on batches of the material as bought from the market. The average moisture content was 68·6% since the dry powder had a moisture content of 3·55% the values obtained for phytin were corrected by 59·5% so as to express them on wet basis.

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TABLE II

The phosphorus distribution of garlic

Form of P	Sample I	Sample II	Sample III	Mean
Total P	0·240	0·236	0·231	0·236
Results expressed as percentages of total P				
Acid soluble P	60·41	58·18	55·84	58·14
Total acid-insoluble P ..	39·59	41·82	44·16	41·86
Total barium-precipitable P .	30·83	32·07	33·01	31·97
Inorganic P	1·63	1·91	1·74	1·77
Hydrolysable P	0·29	0·30	0·30	0·30
Resistant (by difference) P	28·92	29·87	30·22	29·67
Total barium-soluble P ..	9·12	10·97	11·13	10·40
Hydrolysable P	2·79	4·16	5·94	3·63
Resistant (by difference) P	6·23	6·81	7·19	6·78
Phytin P	30·10	30·60	30·50	30·40

From Table II, it is clear that the labile esters like the adenyl pyro-phosphate are present in negligible amounts. The values obtained for the barium-precipitable resistant phosphorus and phytin phosphorus are almost close. It is well known that phytin is precipitated by alkaline earth metals (Arney, 1939) in faintly alkaline medium. Therefore the values obtained for barium-precipitable phosphorus represent the phytin-phosphorus. The barium-soluble fraction is composed of 35% of a labile form of phosphorus compound, the nature of which is not known, and the rest is the *resistant* hexose mono-phosphate. Further experiments are in progress on the nature of the barium-soluble labile compounds.

In Table III the phosphorus distribution of garlic is compared with hose of cereals, vegetables, pulses and condiments.

TABLE III

Phosphorus distribution of garlic
compared with cereals, pulses, vegetables and condiments

No.	Name of foodstuff	Botanical name	Total P %	Phytin P as % of total P	Available non-phytin P as % of total P
	CEREALS[1]				
1	Cholam	*Sorghum vulgare*	0·374	60·20	39·80
2	Rice	*Oryzæ sativa*	0·354	57·70	42·30
	PULSES[1]				
3	Bengal gram ..	*Cicer arietinum*	0·371	60·10	39·90
4	Soya bean ..	*Glycina hispida*	0·832	33·40	66·60
	VEGETABLES[1]				
5	Lady's fingers ..	*Hibiscus esculantus*	0·080	Nil	100·00
6	Snake gourd ..	*Trichosanthes anguina*	0·029	..	100·00
	CONDIMENTS[2]				
7	Chillies (green) ..	*Capsicum annuum*	0·079	4·31	95·70
8	Corriander seeds ..	*Corriandrum sativum*	0·416	77·02	22·98
9	Pepper	*Piper nigrum*	0·197	58·42	41·58
10	Cumin	*Cuminium cynurium*	0·460	33·29	66·71
11	Garlic[3]	*Allium sativum*	0·260	30·50	69·50

[1] Giri (1938);   [2] Sundararajan (1938);   [3] Found.

From Table III it is evident that the condiments occupy an intermediate place for their phytin phosphorus contents between the cereals and pulses and the vegetables.   Garlic compares favourably with Cumin for its phytin phosphorus content.

Summary

1.   Garlic contains 30·5% of the total phosphorus as phytin,   which forms the major fraction of the barium-precipitable acid-soluble phosphorus.

2.   The barium-soluble fraction of the acid-soluble phosphorus is largely composed of the resistant hexose mono-phosphate 65%, and labile phosphorus compounds 35% whose nature is not known.

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ON THE OCCURRENCE OF *MICROSPIRA AESTUARII* IN THE BUCKINGHAM CANAL AT MADRAS

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During the hot weather and south-west monsoon seasons, an offensive odour of sulphuretted hydrogen emanates from the Buckingham Canal, especially in the neighbourhood of the Basin Bridge Railway Station. It once became very acute towards the middle of August 1934. The following experimental investigations were carried out to elucidate the causes of this phenomenon:

(*i*) Experiments were done with Beijerinck's (1900) protein-free sterilised medium in four sets of two bottles each of 250 c.c. capacity; and to each set was added respectively (*a*) 50 c.c. of Buckingham Canal water from a place (S_4) in the Canal where the odour was most intense; (*b*) a few grams of mud from the bottom of the Canal at S_4; and (*c*) same as (*a*) but the contents were sterilised. One set was kept as control. The bottles were set aside at room temperature for a week. At the end of the period, it was found that the contents of the bottles which had been inoculated with the unsterilised Canal water and mud had turned black, while the remaining bottles were colourless. It was therefore clear that the production of sulphuretted hydrogen was of biological origin.

(*ii*) Next, experiments were done with Van Delden's sterilised medium (1904) used for culturing *Microspira æstuarii*. Nine bottles of 250 c.c. capacity containing the above medium were inoculated with 10 c.c. of each of the sample of Canal water taken from nine different places along the Canal and a tenth bottle was kept as control. A second similar set containing the sterilised medium was inoculated with a few grams of bottom mud from each of the nine places mentioned above. The two series of experiments were repeated every month for a year. The contents of all the bottles except the control turned black after a week, thereby showing that (*a*) organisms capable of producing sulphuretted hydrogen were present in all samples throughout the year, and (*b*) H_2S might have been produced by organisms capable of reducing sulphates, sulphites, thiosulphates or of albuminous substances.

(*iii*) Bottom mud from S_4 was inoculated into three bottles half filled with the sterilised Van Delden's medium. The bottles were plugged with

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cotton. Three other bottles which had been similarly treated were completely filled with the medium and were carefully stoppered. All the bottles were incubated at room temperature, and the half filled bottles were shaken every now and then, while the others were left undisturbed, for a week. A control was kept in each case. It was found that while the bottles which were completely filled turned black and contained large quantities of H_2S, the other set of bottles showed a slight blackening at the bottom only and did not evolve any smell of H_2S. These experiments showed that anærobic conditions were essential for the reduction of sulphates.

About 1 c.c. of the bottom liquid from each of the bottles in experiment (3) was inoculated into each of three bottles containing Van Delden's medium and then incubated at room temperature. The contents became hazy on the second day, blackened on the third and became jet black at the end of a week. There was evolution of H_2S also. It was apparent that H_2S had originated from sulphates as there was no other source of sulphur in the liquid medium used.

(*iv*) The sulphates were eliminated from a sample of the Canal water in the usual way. To lots of three bottles each of 250 c.c. capacity containing 200 c.c. of the sulphate-free Canal water were added respectively.

(*a*) 50 c.c. of sterilised glucose bouillon.

(*b*) 50 c.c. of sterilised glucose bouillon + a few grams of bottom mud from S_4.

(*c*) 50 c.c. of sterilised glucose bouillon + 0·1% ammonium sulphate.

To another set of three lots of three bottles each containing 200 c.c. of Canal water from S_4 were added respectively:

(*d*) 50 c.c. of sterilised glucose bouillon.

(*e*) 50 c.c. of sterilised glucose bouillon + a few grams of pasturised mud from S_4.

(*f*) 50 c.c. of sterilised glucose broth + a few grams of bottom mud from S_4.

The first set of three bottles were completely filled with the sulphate-free Canal water while the last set with the Canal water; all of them were well stoppered and kept aside at room temperature for one week at the end of which period they were examined for H_2S. H_2S was not detected in (*a*), (*b*), (*c*) and (*e*) while it was found only in one of the bottles of (*d*) and in all bottles of (*f*). So it was concluded that (*i*) sulphates were necessary

for sulphate reduction and (*ii*) sulphate reducing organisms were always present in the bottom mud of the Canal.

(*v*) Next, H_2S was estimated according to the methods followed by Beijerinck and Van Delden using sterilised Van Delden's medium for *Microspira æstuarii*. Inoculations were made from cultures showing H_2S. The results are shown in the table below.

TABLE I

Period of incubation	No. of days	Composition of liquid		H_2S. in mg./1
March 20th to April 1st, 1935	11	Buckingham Canal water +Bottom mud from	S_4	113·5
,, ,,	,,	,, ,,	S_5	100·6
,, ,,	,,	,, ,,	S_8	89·4
,, ,,	,,	,, ,,	S_9	98·2
March 20th to May 13th, 1935	53	,, ,,	S_4	177·4
April 12th to April 29th, 1935	17	Van Delden's medium for *Microspira æstuarii*		485·3
March 20th to May 13th, 1935	53	,,		521·0
,, ,,	,,	,,		777·6
March 25th to May 13th, 1935	49	,,		764·2
,, ,,	,,	,,		873·2
April 1st to May 13th, 1935	44	,,		685·2
,, ,,	,,	,,		847·2
,, ,,	,,	,,		911·1
,, ,,	,,	,,		1016·0
April 5th to May 13th, 1935	38	,,		431·3
,, ,,	,,	,,		661·9
April 30th to May 13th, 1935	31	,, + 10 c.c. of B. Canal water from	S_4	564·4
,, ,,	,,	,, ,,	S_1	24·5
,, ,,	,,	,, ,,	S_3	160·5
,, ,,	,,	,, ,,	S_8	58·5
,, ,,	,,	,, ,,	S_9	67·3

S_4: From the wharf opposite to the entry of the Otteri Nullah into the Buckingham Canal.

From the above table, it will be seen that as much as 1016 milligrams per litre of sulphuretted hydrogen have been produced from sulphates by organisms present in the Canal water.

(*vi*) Experiments similar to those of Van Delden were done with peptone, sodium acetate, sodium succinate, sodium citrate, aspargin, sodium malate, sodium taurocholate and sodium lactate; and it was found that all the above substances were found very useful as organic nutrient substances for sulphate reduction.

To obtain a pure culture of the sulphate reducing organism the method adopted was almost similar to that of Dey and Ganapati (1934) used for the isolation of *Spirillum desulfuricans* from the Madras slow sand filters. Microscopic examination of the black agar colonies which were easily stained by alcoholic gentian violet showed that they consisted of " short very little twisted spirilla " almost resembling *Spirillum desulfuricans* isolated from the Madras filters. The organism measured about $3\,\mu$ long $1\,\mu$ broad. It resembled *Microspira æstuarii* of Van Delden in that it was always accompanied by a micrococcus in all cultures.

That the coccus which invariably accompanied the spirillum was not responsible for sulphate reduction was shown by spreading a drop of the liquid culture over a sterilised nutrient agar plate and in which it was found to grow very well. By repeated subcultures both in agar slants and in broth tubes, a pure culture of the micro-coccus was obtained. Portions of an agar colony and also a few drops from the nutrient broth were transferred to bottles containing sterilised Van Delden's liquid medium for *Microspira æstuarii*. The bottles were completely filled with the medium, stoppered and set aside for one week, at the laboratory temperature. The bottles did not turn hazzy or black, thereby showing that the coccus was incapable of reducing sulphates to H_2S.

By repeated sub-cultures, a pure culture of the spirillum also was obtained. The purity of the liquid culture was tested by spreading a drop of the liquid into an ordinary nutrient agar plate and there was no growth after 24 hours incubation at $37°$ C. (Van Delden, *loc. cit*).

Sulphate reduction with pure culture

In order to confirm that the isolated organism was the sulphate reducing *Microspira æstuarii*, 0.5 c.c. of the pure liquid culture was inoculated into Van Delden's sterilised culture medium using different concentrations of sodium chloride as shown in the following table. The amount of H_2S formed was quantitatively estimated, as before.

TABLE II

Table showing sulphate reduction with a pure culture of Microspira æstuarii

Culture	Composition of liquid medium		Period of incubation	No. of days	H_2S in mg./1
I	V. D. medium + 0 % NaCl		August 12th to September 11th 1935	30	0
II	,,	+ 0·5 ,,	,,	,.	73·5
III	,,	+ 1·0 ,,	,,	,,	359·5
IV	,,	+ 1·5 ,,	,,	,,	402·8
V	,,	+ 2·0 ,,	,,	,,	402·8
VI	,,	+ 2·5 ,,	,,	,,	395·9
VII	,,	+ 3·0 ,,	,,	,,	390·6
VIII	,,	+ 3·5 ,,	,,	,,	390·6
IX	,,	+ 4·0 ,,	,,	,,	374·2

It will be seen from the above table that the organism isolated from the Canal was able to produce maximum amount of H_2S at a concentration of 1·5 to 2·5% of salt and that it was not able to produce H_2S in its absence. So, the sulphate reducing organism isolated from the Buckingham Canal is the same as or is very similar to that isolated by Van Delden from sea water, *viz.*, *Microspira æstuarii*.

(*a*) Experiments relating to the presence of organisms in the Canal, capable of producing H_2S from albuminous substances and sulphur containing inorganic substances other than sulphates.

Melted agar tubes containing Tilley's (1923) medium were inoculated with the diluted water samples from stations S_4, S_5, S_6, S_7 and S_8 (different places along the Buckingham Canal) and the inoculated agar tubes were poured carefully into sterile Petri dishes. One set of tubes was incubated aerobically and the other set anærobically in desiccators containing alkaline pyrogallol. All the plates were incubated at 37° C., for 48 hours. In both cases, numerous black colonies were obtained which on microscopic examination were found to consist chiefly of bacilli and a few of cocci.

Every time, eight pure cultures of the bacilli and two of the cocci were inoculated into bottles containing Beijerinck's (1900) protein-free medium. Within 24 hours a marked fermentation was noticed, but no production of

H₂S in all the bottles even after a week's incubation at the room tempera-ture.

On the other hand, when the sulphate was replaced by an equivalent quantity of sodium thiosulphate, sulphite or flowers of sulphur in the medium, and the same organisms were inoculated into bottles containing the latter medium, H₂S was produced in each case. These experiments showed, therefore, that the bacilli and cocci which were also present in the Canal water were capable of reducing all sulphur containing substances other than sulphates.

Pure cultures of the coli group of organisms were isolated from the Buckingham Canal water according to the usual technique followed in the bacteriological examination of waters. They were not able to reduce sulphates to H₂S in the Beijerinck's (1895) or Van Delden's medium (*loc. cit.*) but they were able to produce H₂S if the sulphates were replaced by sodium thiosulphate or flowers of sulphur.

Thus the existence of the two groups of organisms in the Buckingham canal is established. But the production of H₂S due to reduction of sulphates by *Microspira æstuarii* appears to be a major process, as conditions more favourable for its activity are present in the Buckingham Canal.

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